Hybrid Multi-Mode Microplate Reader

Synergy™ Neo2

Operator's Manual

BioTek®

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Operation Manual

knowledge

Action movie

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Synergy™ Neo2

Hybrid Multi-Mode Microplate Reader

Operator's Manual

BioTek® Instruments, Inc.
August 2016

PN 1351000
Revision B

Notices

BioTek® Instruments, Inc.

Highland Park, P.O. Box 998
Winooski, Vermont 05404-0998 USA

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Contact Information

BioTek® Instruments, Inc.

Highland Park, P.O. Box 998

Winooski, Vermont 05404-0998 USA

Global Service and Support

BioTek instrument service and repair is available worldwide at one of BioTek's International Service Centers and in the field at your location. To arrange for service or repair of your instrument, contact the office nearest you; visit www.biotek.com for up-to-date contact information. For customer service, sales, and technical assistance, refer to the information below.

Customer Service and Sales

Internet:	www.biotek.com
Phone:	888-451-5171 (toll-free in the U.S.)
	802-655-4740 (outside the U.S.)
Fax:	802-655-7941
Email:	customercare@biotek.com

Service/Technical Assistance Center (TAC)

Phone:	800-242-4685 (toll-free in the U.S.)
	802-655-4740 (outside the U.S.)
Fax:	802-654-0638
Email:	tac@biotek.com

European Coordination Center/Authorized European Representative

BioTek® Instruments GmbH

Kocherwaldstrasse 34

D-74177 Bad Friedrichshall

Germany

Internet:	www.biotek.de
Phone:	+49 (0) 7136 9680
Fax:	+49 (0) 7136 968 111
Email:	info@biotek.de

Revision History

Rev	Date	Changes
A	7/2015	Initial release to production
B	8/2016	*General:* Added support for the FluorescenceTest Plate PN 1400006 *Preface:* Changed the CE information to reflect current CE directive titles. *Chapter 1, Introduction:* Added information about the Absorbance Test Plate PN 7260551 to the Optional Accessories, updated the "Materials for Conducting Liquid Tests" chart. *Chapter 2, Installation:* Reworded "13. Configure Dispensers" to "13. Set Dispenser Calibration Values." *Chapter 3, Getting Started:* Replaced table showing the various modules with tables from Marketing, added a note to the "Injectors" section about referring to the *Injection System—Chemical Compatibility Technical Note*. *Chapter 4, Preventive Maintenance and Chapter 5, As-Needed Maintenance:* Updated text to add wording about wringing out the cleaning cloth so that it is not dripping wet before wiping the instrument. *Chapter 6, Instrument Qualification:* Added information about using the Absorbance Test Plate PN 7260551; updated R Square values to include four digits to the right of the decimal point; for the FP Test materials, clarified that the Invitrogen kit includes two, unused red polarization solutions. For the TRF materials, corrected the notation for the Upper Top Filter Cube (dual PMT instruments). For the [SF] filter-based Sensitivity Test pass/fail criteria, corrected the mismatch between the DL column header and the Top/Bottom criteria. *Appendix D:* Added new appendix with sample System Test and Absorbance Plate Test reports.

Document Conventions

	This icon calls attention to important safety notes.
Warning!	A Warning indicates the potential for bodily harm and tells you how to avoid the problem.
Caution	A Caution indicates potential damage to the instrument and tells you how to avoid the problem.
Note	Bold text is primarily used for emphasis.
italic	Topics that apply only to specific Synergy Neo2 models are proceeded by a notice in italic, for example, *Applies only to Synergy Neo2 models with injectors*.
	This icon calls attention to important information.

Intended Use Statement

The Synergy Neo2 is a hybrid multimode microplate reader. The performance characteristics of the data reduction software have not been established with any laboratory diagnostic assay. The user must evaluate this instrument and PC-based software in conjunction with their specific assay(s). This evaluation must include the confirmation that performance characteristics for the specific assay(s) are met.

- BioTek Gen5 software package provides the user with instrument control and data reduction capabilities.
- The Synergy Neo2 can operate with standard robotic systems, such as the Synergy Neo Stacker and BioStack 4.
- If the instrument has an "IVD" label, it may be used for clinical and nonclinical purposes, including research and development. If there is no such label, the instrument may be used only for research and development or other non-clinical purposes.

Quality Control

It is considered good laboratory practice to run laboratory samples according to instructions and specific recommendations included in the assay package insert for the test to be conducted. Failure to conduct Quality Control checks could results in erroneous test data.

Warranty and Product Registration

Please take a moment to review the warranty information that shipped with your product. Please also register your product with BioTek to ensure that you receive important information updates about the product(s) you have purchased.

You can register online through the Customer Resource Center (CRC) at www.biotek.com or by calling 888-451-5171 or 802-655-4740.

Warnings

Operate the instrument on a level, stable surface away from excessive humidity.

Bright light can reduce the linear performance range of the instrument.

Measurement values may be affected by extraneous particles (such as dust) in the microplate wells. A clean work area is necessary to ensure accurate readings.

When operated in a safe environment according to the instructions in this document, there are no known hazards associated with the instrument. However, the operator should be aware of certain situations that could result in serious injury; these may vary depending on the instrument model. See Hazards and Precautions.

Hazards

The following hazards are provided to help avoid injury:

Warning! Power Rating. The instrument's power supply or power cord must be connected to a power receptacle that provides voltage and current within the specified rating for the system. Use of an incompatible power receptacle may produce electrical shock and fire hazards.

Warning! Electrical Grounding. Never use a plug adapter to connect primary power to the external power supply. Use of an adapter disconnects the utility ground, creating a severe shock hazard. Always connect the power cord directly to an appropriate receptacle with a functional ground.

Warning! Internal Voltage. Always turn off the power switch and unplug the power supply before cleaning the outer surface of the instrument or removing its top case.

Warning! Liquids. Avoid spilling liquids on the instrument; fluid seepage into internal components creates a potential for shock hazard. If a spill occurs while a program is running, abort the program and turn off the instrument. Wipe up all spills immediately. Do not operate the instrument if internal components have been exposed to fluid. Contact BioTek TAC for assistance.

Warning! Potential Biohazards. Some assays or specimens may pose a biohazard. Adequate safety precautions should be taken as outlined in the assay's package insert. Always wear safety glasses and appropriate protective equipment, such as chemical-resistant rubber gloves and apron.

Warning! Unspecified Use. Failure to operate this equipment according to the guidelines and safeguards specified in this manual could result in a hazardous condition.

Warning! Software Quality Control. The operator must follow the manufacturer's assay package insert when modifying software parameters and establishing reading methods. Failure to conduct quality control checks could result in erroneous test data.

Warning! Reader Data Reduction Protocol. No limits are applied to the raw measurement data. All information exported via computer control must be thoroughly analyzed by the operator.

Warning! Laser Beam. Serious eye injury may occur if you stare directly into the laser beam of the barcode scanner during operation of the scanner. This hazard is noted by the symbol shown here. Do not look directly into the laser beam during operation of the scanner.

Warning! Service. Only qualified technical personnel should perform service procedures on internal components.

Warning! Accessories. Only accessories that meet the manufacturer's specifications shall be used with the instrument.

Warning! Pinch Hazard. Some areas of the instrument or its components can present pinch hazards when the instrument is operating. These areas are marked with the symbol shown here. Keep hands/fingers clear of these areas when the instrument is operating.

Warning! The instrument with all available modules weights up to **85 lbs. (38.6 kg)**. Use two people when lifting and carrying the instrument.

Precautions

The following precautions are provided to help avoid damage to the instrument:

Caution: Service. The instrument should be serviced by BioTek-authorized service personnel. Only qualified technical personnel should perform troubleshooting and service procedures on internal components.

Caution: Spare Parts. Only approved spare parts should be used for maintenance. The use of unapproved spare parts and accessories may result in a loss of warranty and potentially impair instrument performance or cause damage to the instrument.

Caution: Environmental Conditions. Do not expose the system to temperature extremes. For proper operation, ambient temperatures should remain within the range listed in Appendix A, Specifications. Performance may be adversely affected if temperatures fluctuate above or below this range. Storage temperature limits are broader.

Caution: Sodium Hypochlorite. Do not expose any part of the instrument to the recommended diluted sodium hypochlorite solution (bleach) for more than 20 minutes. Prolonged contact may damage the instrument surfaces. Be certain to rinse and thoroughly wipe all surfaces.

Caution: Power Supply. Use only the power supply shipped with the instrument. Operate this power supply within the range of line voltages listed on it.

Caution: Shipping Hardware. The carrier shipping screw and filter reader shipping bracket must be removed before operating the instrument. They must be reinstalled before shipping the instrument. Please retain these components in the event the instrument needs to be returned to BioTek. See **Chapter 2, Installation**.

Caution: Disposal. Dispose of the instrument according to Directive 2012/19/EU, "on waste electrical and electronic equipment (WEEE)" or local ordinances.

Caution: Warranty. Failure to follow preventive maintenance protocols may void the warranty. See **Chapter 5, Preventive Maintenance**.

Caution: Electromagnetic Environment. Per IEC 61326-2-6 it is the user's responsibility to ensure that a compatible electromagnetic environment for this instrument is provided and maintained in order that the device will perform as intended.

Caution: Electromagnetic Compatibility. Do not use this device in close proximity to sources of strong electromagnetic radiation (e.g., unshielded intentional RF sources), because these may interfere with the proper operation.

Caution: Barcode Scanner Mirror. Do not scratch or damage the mirror when unpacking or installing the barcode scanner.

CE Mark

Based on the testing described below and information contained herein, this instrument bears the CE mark

Refer to the Declaration of Conformity for specific details.

Directive 2014/30/EU: Electromagnetic Compatibility

Emissions—Class A

The system has been type-tested by an independent, accredited testing laboratory and found to meet the requirements of EN 61326-1: Class A for Radiated Emissions and Line Conducted Emissions.

Verification of compliance was conducted to the limits and methods of EN 55011 – (CISPR 11) Class A. In a domestic environment it may cause radio interference, in which case you may need to mitigate the interference.

Immunity

The system has been type-tested by an independent, accredited testing laboratory and found to meet the requirements of EN 61326-1 and EN 61326-2-6 for Immunity. Verification of compliance was conducted to the limits and methods of the following:

EN 61000-4-2, Electrostatic Discharge Immunity
EN 61000-4-3, Radiated Radio Frequency (RF) Immunity
EN 61000-4-4, Electrical Fast Transient/Burst Immunity
EN 61000-4-5, Surge Immunity
EN 61000-4-6, Conducted Radio Frequency Immunity
EN 61000-4-8, Powerline Magnetic Field Immunity
EN 61000-4-11, Voltage Dips and Short Interrupts Immunity

Directive 2014/35/EU Low Voltage (Safety)

The system has been type-tested by an independent testing laboratory and was found to meet the requirements of this Directive. Verification of compliance was conducted to the limits and methods of the following:

EN 61010-1. "Safety requirement for electrical equipment for measurement, control and laboratory use. Part 1, General requirements."
EN 61010-2-081. “Particular requirements for automatic and semi-automatic laboratory equipment for analysis and other purposes.”
EN 61010-2-010. “Particular requirements for laboratory equipment for the heating of materials.“

Directive 2012/19/EU: Waste Electrical and Electronic Equipment

Disposal Notice: Dispose of the instrument according to Directive 2012/19/EU, “on waste electrical and electronic equipment (WEEE)” or local ordinances.

Directive 98/79/EC: In Vitro Diagnostics (if labeled for this use)

- Product registration with competent authorities
- EN 61010-2-101. “Particular requirements for in vitro diagnostic (IVD) medical equipment.”
- Traceability to the U.S. National Institute of Standards and Technology (NIST).

Electromagnetic Interference and Susceptibility

USA FCC CLASS A

RADIO AND TELEVISION INTERFERENCE

NOTE: This equipment has been tested and found to comply with the limits for a Class A digital device, pursuant to Part 15 of the FCC Rules. These limits are designed to provide reasonable protection against harmful interference when the equipment is operated in a commercial environment. This equipment generates, uses, and can radiate radio frequency energy and, if not installed and used in accordance with the instruction manual, may cause harmful interference to radio communications. Operation of this equipment in a residential area is likely to cause harmful interference, in which case the user will be required to correct the interference at their own expense.

In order to maintain compliance with FCC regulations, shielded cables must be used with this equipment. Operation with non-approved equipment or unshielded cables is likely to result in interference to radio and television reception.

Canadian Department of Communications Class A

This digital apparatus does not exceed Class A limits for radio emissions from digital apparatus set out in the Radio Interference Regulations of the Canadians Department of Communications.

Le present appareil numerique n'emet pas du bruits radioelectriques depassant les limites applicables aux appareils numerique de la Class A prescrites dans le Reglement sur le brouillage radioelectrique edicte par le ministere des Communications du Canada.

User Safety

This device has been type-tested by an independent laboratory and found to meet the requirements of the following:

- **Underwriters Laboratories UL 61010-1**

  "Safety requirements for electrical equipment for measurement, control and laboratory use; Part 1: general requirements"

- **Canadian Standards Association CAN/CSA C22.2 No. 61010-1**

  "Safety requirements for electrical equipment for measurement, control and laboratory use; Part 1: general requirements"

For International User Safety requirements, see page xiii.

Safety Symbols

Some of the following symbols may appear on the instrument or accessories:

Alternating current

Courant alternatif

Wechselstrom

Corriente alterna

Corrente alternata

Direct current

Courant continu

Gleichstrom

Corriente continua

Corrente continua

Both direct and alternating current

Courant continu et courant alternatif

Gleich - und Wechselstrom

Corriente continua y corriente alterna

Corrente continua e corrente alternata

Earth ground terminal

Borne de terre

Erde (Betriebserde)

Borne de tierra

Terra (di funzionamento)

Warning, risk of crushing or pinching

Attention, risque d'écrasement et pincement

Warnen, Gefahr des Zerquetschens und Klemmen

Precaución, riesgo del machacamiento y sejeción

Attenzione, rischio di schiacciare ed intrappolarsi

Warning, hot surface

Attention, surface chaude

Vorsicht, heiße Oberfläche

Precaución, superficie caliente

Attenzione, superfice calda

Laser radiation: Do not stare into beam

Rayonnement laser: Ne pas regarder dans le faisceau

Laserstrahlung: nicht in den strahl blicken

Radiación de laser: No mire fijamente al rayo

Radiazione di laser: Non stare nel fascio

Warning, potential biohazards

Attention, risques biologiques potentiels

Warnung! Moegliche biologische Giftsoffe

Atención, riesgos biológicos

Attenziones, rischio biologico

Protective conductor terminal

Borne de terre de protection

Schultzleiteranschluss

Borne de tierra de protección

Terra di protezione

On (Supply)

Marche (alimentation)

Ein (Verbindung mit dem Netz)

Conectado

Chiuso

Off (Supply)

Arrêt (alimentation)

Aus (Trennung vom Netz)

Desconectado

Aperto (sconnessione dalla rete di alimentazione)

Warning, risk of electric shock

Attention, risque de choc électrique

Gefährliche elektrische schlag

Precaución, riesgo de sacudida eléctrica

Attenzione, rischio di scossa elettrica

Caution (refer to accompanying documents)

Attention (voir documents d'accompanement)

Achtung siehe Begleitpapiere

Atención (vease los documentos incluidos)

Attenzione, consultare la doc annessa

Consult instructions for use

Consulter la notice d'emploi

Gebrauchsanweisung beachten

Consultar las instrucciones de uso

Consultare le istruzioni per uso

In vitro diagnostic medical device

Dispositif médical de diagnostic in vitro

Medizinisches In-Vitro Diagnostikum

Dispositivo médico de diagnóstico in vitro

Dispositivo medico diagnostico in vitro

Separate collection for electrical and electronic equipment

Les équipements électriques et électroniques font l'objet d'une collecte sélective

Getrennte Sammlung von Elektro- und Elektronikgeräten

Recogida selectiva de aparatos eléctricos y electrónicos

Raccolta separata delle apparecchiature elettriche ed elettroniche

Chapter 1

Introduction

This chapter introduces the Synergy Neo2 Hybrid Multi-Mode Microplate Reader, describes its hardware and software features, and provides contact information for technical assistance.

Product Description

The Synergy Neo2 is a hybrid multi-mode microplate reader. Depending on the model, Synergy Neo2 detection modes include fluorescence intensity (FI), fluorescence polarization (FP), time-resolved fluorescence (TRF), luminescence, UV-visible absorbance, and alpha. The reader is modular, and upgrade options are available; contact BioTek Customer Care for more information.

The reader is computer-controlled using Gen5 software for all operations, including data reduction and analysis. The Synergy Neo2 is robot accessible and compatible with the BioTek Synergy Neo Stacker and BioStack 4. Gen5 supports OLE automation to facilitate the Synergy Neo2's integration into an automated system.

The Synergy Neo2 can perform reads using barcoded filter cubes or a monochromator.[1] The filter-based system can perform top and bottom fluorescence, luminescence, and alpha reads. Filter fluorescence uses a xenon flash light source, along with interference filters and dichroic mirrors for wavelength specificity and up to three photomultiplier tubes (PMTs). To run a fluorescence polarization protocol, the filter cube must contain polarizing filters. Luminescence is measured through an empty filter position in the filter cube; filters can be used if light filtering is necessary.

The monochromator-based system, which has both top and bottom probes, is used for absorbance, fluorescence, and luminescence spectral reads. The xenon lamp allows for both UV and visible light measurements. The monochromator provides wavelength selection from 230–999 nm in 1 nm increments. Available absorbance and fluorescence read methods are endpoint, area scan, spectral scanning, and pathlength correction. You can use the monochromator optics for luminescence spectral scanning.

The alpha detection method can be used for endpoint reads using the top filter system in non-synchronized plate mode.

The Synergy Neo2 has 4-Zone temperature control from 4°C over ambient to 65°C. (For alpha detection mode, the range is 3°C over ambient to 30°C.) Internal plate shaking, with both linear and orbital modes, is supported to ensure that reagents are properly mixed prior to reading.

The Synergy Neo2 supports the reading of 6-, 12-, 24-, 48-, 96-, 384, and 1536-well microplates with 128 x 86 mm geometry as well as the Take3 and Take3 Trio Multi-Volume Plates.

> Use of microplates other than those listed here can result in positioning errors during program execution.

Models with injectors support dual-reagent dispensing to 6-, 12-, 24-, 48-, 96-, and 384-well microplates. An external dispense module pumps fluid from the supply bottles to the two injectors located inside the instrument.

[1] This dual light path capability is protected by U.S. patent number 8,218,141.

See **Appendix A** for performance and technical specifications.

Package Contents

Package contents and part numbers are subject to change. Please contact BioTek Customer Care with any questions.

Item	Part #
Synergy Neo2 Operator's Manual	1351000
Power cord set (specific to installation environment):	
Europe (Schuko)	75010
USA/International	75011
United Kingdom	75012
Australia/New Zealand	75013
USB cable	75108
Filter cube rack	1030541
Plastic storage bag and Velcro strips	
Models with an external dispense module (packed separately), with the following accessories:	
Injector	8040541
Inlet tubes (2) from supply bottles to syringe drives	7082121
250-µL syringes (2)	7083000
Syringe thumbscrews	19511
Priming plate	8042202
Injector tip priming trough	8042068
Dispense module communication cable	75107
Dispense module front cover	8042313
Dispense module box	8040579
Supply bottles (2, 30 mL)	7122609
Supply bottle holders (2)	8042193
Injector tip cleaning stylus and plastic storage bag	2872304
Strap reagent racks (6)	7212035

Optional Accessories

Accessory availability and part numbers are subject to change. Please contact BioTek Customer Care if you have any questions or visit www.biotek.com and use the Accessories search tool.

Item	Part #
7-filter Absorbance Test Plate for absorbance measurement testing	7260522
Absorbance Test Plate for absorbance measurement testing at 340 nm	7260551
Synergy Neo2 Product Qualification (IQ-OQ-PQ) package	1350526
Microplate Barcode Scanner	1030008
Take3 Micro-Volume Plate	TAKE3
Take3 Trio Micro-Volume Plate	TAKE3TRIO
RS-232 serial cable	75034
PCR Tube Adapter Plates	6002072 6002076
BioCell Quartz Vessel	7272051
BioCell Adapter Plate	7270512
Replacement Shipping Materials	1033027 (instrument shipping box), 1033044 (accessories shipping box) 1030539 (carrier shipping bracket [1]) 1032190 (carrier shipping bracket screws [2]) 1030527 (filter chamber shipping hardware [3])
Fluorescence Test Plate	1400006
Luminometer Reference Microplate (includes microplate carrier adapter PN #8042263 for Synergy Neo2)	8030015
Gas controller, CO_2 control only	1210012
Gas controller, CO_2 and O_2 control	1210013
Filter cubes	Contact BioTek or visit our website for part numbers and availability

The Synergy Neo2 is compatible with the Synergy Neo Stacker and BioStack 4. The stacker rapidly and systematically transfers microplates to and from the instrument's microplate carrier. Contact BioTek or visit our website to learn more.

Materials for Conducting Liquid Tests

Manufacturer part numbers are subject to change.

Item	Part Number
Absorbance Liquid Tests	
BioTek Wetting Agent Solution	BTI #7773002
BioTek QC Check Solution #1 (25 mL)	BTI #7120779
BioTek QC Check Solution #1 (125 mL)	BTI #7120782
Phosphate-Buffered Saline (PBS) tablets (pH 7.2–7.6)	Sigma #P4417
β-NADH Powder (β-Nicotinamide Adenine Dinucleotide, reduced form)	BTI #98233 or Sigma #N6785-10VL
Fluorescence Liquid Tests	
Test Kits	
Kit with microplates and test solutions for conducting Corners/Sensitivity/Linearity (FI) tests using Sodium Fluorescein and Methylumbelliferone, and Time-Resolved Fluorescence (TRF) tests using Europium	BTI #7160010 (contains 7160013, 7160012, and 7160011 described below)
Kit for FI tests using Sodium Fluorescein	BTI #7160013
Kit for FI tests using Methylumbelliferone	BTI #7160012
Kit for TRF tests using Europium	BTI #7160011
Kit for Fluorescence Polarization (FP) test	BTI #7160014 or Invitrogen #P3088
Individual Materials	
Sodium Fluorescein Powder, 1 mg vial	BTI #98155
Methylumbelliferone, 10 mg vial	BTI #98156
Carbonate-Bicarbonate Buffer (CBB) capsules	Sigma #3041

Item	Part Number
Phosphate-Buffered Saline (PBS) tablets, pH 7.2–7.6	Sigma #P4417
Sodium Borate, pH 9.18	Fisher Scientific #159532 or equivalent
Injection System Tests	
BioTek Green Test Dye	BTI #7773003

Product Support and Service

Technical Assistance Center (TAC)

If your instrument or software fail to function properly, if you have questions about how to use or maintain our products, or if you need to send an instrument to BioTek for service or repair, please contact our Technical Assistance Center ("TAC").

TAC is open from 8:30 AM to 5:30 PM (EST), Monday through Friday, excluding standard U.S. holidays.

- **Phone:** (800) 242-4685 or (802) 655-4740
- **Fax:** (802) 654-0638
- **E-Mail:** tac@biotek.com
- **Web:** www.biotek.com

Please be prepared to provide the following information:

- Your name and company information, along with a daytime phone or fax number, and/or an e-mail address
- The product name, model, and serial number
- The instrument software part number and basecode version (available via Gen5 for the Synergy Neo2 by selecting **System > Instrument Control > Information**)
- The version of Gen5. From the main Gen5 screen, select **Help > About Gen5**.
- A copy of the Synergy Neo2 system test results. See page 1.
- For troubleshooting assistance or instruments needing repair, the specific steps that produce your problem and any error codes displayed in Gen5 (see also **Appendix B, Error Codes**)

If you need to return an instrument to BioTek for service or repair, please contact the TAC for a Service Call Notice and the shipping address. Repackage the instrument according to the instructions at the end of **Chapter 2, Installation**.

Applications Support

BioTek's fully equipped Application Laboratory provides our on-staff scientists with the means to assist you with the integration of our instrumentation and software with your unique scientific applications. If you are having difficulty with optimizing fluorescence sensitivity or integrating a unique data reduction transformation, or you are just looking for a recommendation on an appropriate fluorophore, contact us.

Phone: (888) 451-5171 **E-Mail:** applications@biotek.com

Chapter 2

Installation

This chapter includes instructions for unpacking and setting up the Synergy Neo2 and, if applicable, the external dispenser, the barcode reader, and the Synergy Neo Stacker. Instructions are also included for preparing the reader and dispenser for shipment.

Product Registration

Please register your product(s) with BioTek to ensure that you receive important information and updates about the product(s) you have purchased.

Register online through BioTek's Customer Resource Center (CRC) at www.biotek.com or by contacting BioTek Customer Care.

Important Pre-Installation Information

	This chapter contains installation and setup tasks for a Synergy Neo2 reader equipped with all of the available modules. Your Synergy Neo2 may be different. Perform the installation and setup tasks in the order presented, skipping those that do not apply to your reader's configuration. **Materials:** You will need a Phillips screwdriver to perform some of the steps in this section. You will also need a small wrench; this item is supplied with the instrument.
	Remove the shipping hardware before turning on the instrument. Reinstall the shipping hardware before repackaging the instrument for shipping. The carrier shipping bracket should be installed even if moving the instrument from room to room, or to an adjacent building. If the carrier is not secured, it will move as the instrument does, and there is a **chance of damage** to the carrier assembly.
	The instrument with all available modules weighs up to **85 lbs. (38.6 kg)**. Use two people when lifting and carrying the instrument.

1: Unpack and Inspect the Reader

The Synergy Neo2 should be removed from the box by two people. The instrument with all available modules weighs up to **85 pounds (38.6 kg)**.

If the packaging materials are in good condition, keep them in case you need to ship the reader to BioTek for repair or replacement. Using other forms of commercially available packaging, or failing to follow the repackaging instructions, may **void your warranty**. You can order replacement shipping materials (PN 1033027) if necessary. The shipping box, accessories box, foam caps, and so on are included as a whole set under this part number and cannot be ordered separately.

During the unpacking process, inspect the packaging, reader, and accessories for shipping damage. If the reader is damaged, notify the carrier and your BioTek representative. Keep the shipping boxes and the packaging materials for the carrier's inspection. BioTek will arrange for repair or replacement immediately.

1. Open the shipping box, remove the instrument from the box, and place it on a level, stable surface.
2. Place the packaging materials back into the shipping box for reuse if the instrument needs to be shipped again.

2: Unpack and Inspect the Dispenser

Save all packaging materials. If you need to ship the dispenser to BioTek for repair or replacement, you must use the original materials. Using other forms of commercially available packaging, or failing to follow the repackaging instructions, may void your warranty.

During the unpacking process, inspect the packaging, the dispenser, and accessories for shipping damage. If the dispenser is damaged, notify the carrier and your BioTek representative. Keep the shipping boxes and the packaging materials for the carrier's inspection. BioTek will arrange for repair or replacement of your dispense module immediately.

If applicable, perform these steps to unpack the dispenser.

1. Open the shipping box. Remove the accessories box and foam insert that contains the injector tubing and bottle holders.
2. Lift out the dispenser and place it on a level surface.

3. Open the accessories box and remove its contents. The accessories should include the dispenser-related items listed under **Package Contents and Accessories** in Chapter 1.
4. Place all packaging materials into the shipping box for reuse if the dispenser needs to be shipped.

3: Unpack and Inspect the Gas Controller (if applicable)

If applicable, perform these steps to unpack the gas controller.

1. Open the shipping box.
2. Lift out the accessories (power supply, tubing, and manual), and set them aside.
3. Lift out the gas controller, and place it on a level surface.
4. Place all packaging materials into the shipping box for reuse if the gas controller needs to be shipped.

4: Select an Appropriate Location

Install the reader on a level, stable surface in an area where ambient temperatures between 18°C (64°F) and 40°C (104°F) can be maintained.

For alpha laser reads, the ambient temperature should be no warmer than 30°C (86°F).

Leave at least six inches of space between the instrument's rear panel and any other object. This space ensures proper air flow in and out of the instrument.

If you intend to use the bottom filter access door on the left side of the instrument, ensure that you select a location that allows for easy access to the door.

The reader is sensitive to extreme environmental conditions. Avoid the following:

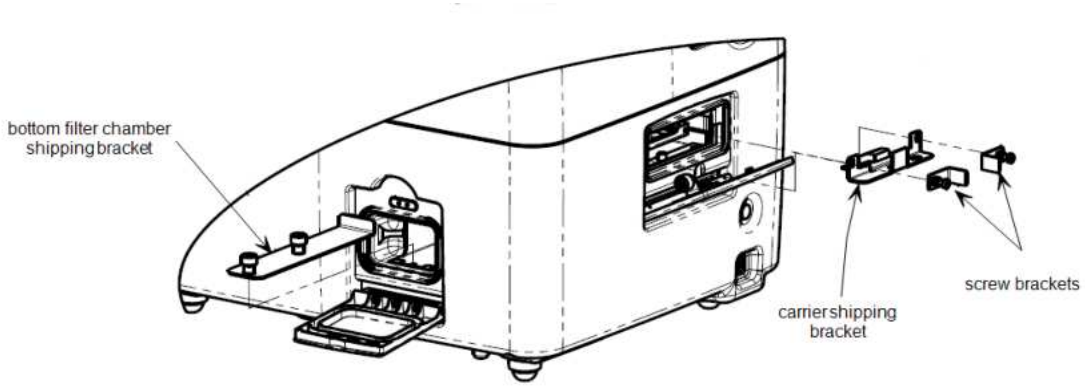
- **Excessive humidity.** Condensation directly on the sensitive electronic circuits can cause the instrument to fail internal self-checks. The humidity must be in the range of 10–85%, non-condensing.
- **Excessive ambient light.** Bright light may affect the reader's optics and readings, reducing its linear range.
- **Dust.** Readings may be affected by extraneous particles (such as dust) in the microplate wells. A clean work area is necessary to ensure accurate readings.

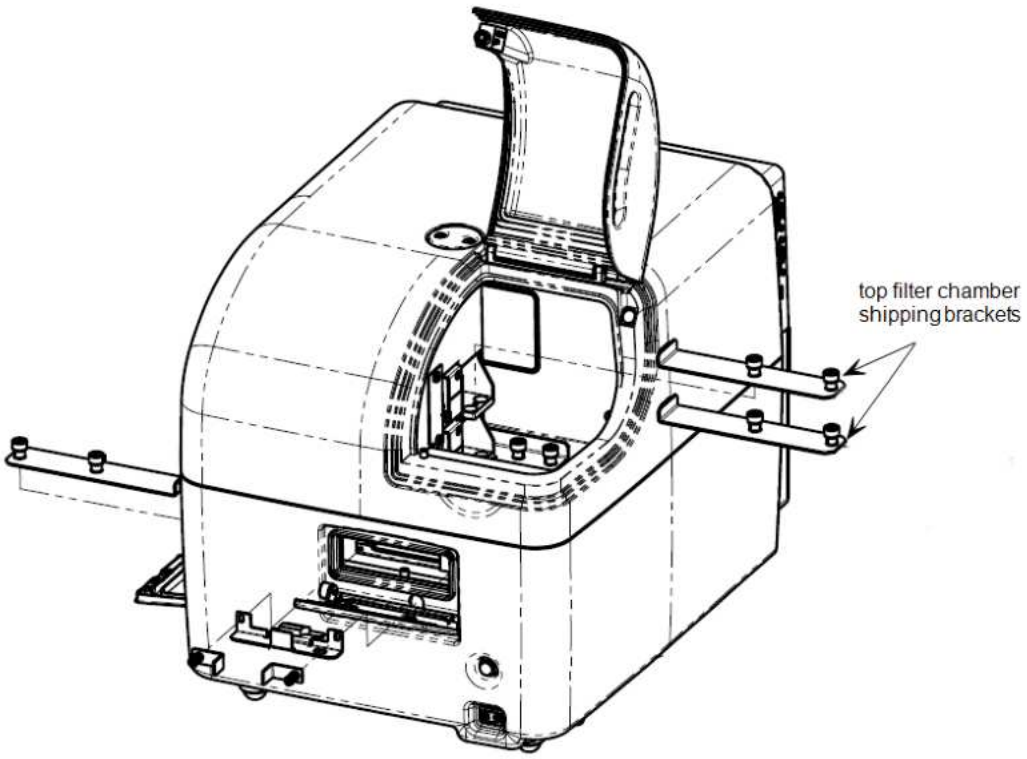
	If you are installing the Synergy Neo Stacker or BioStack 4 for operation with the Synergy Neo2, you may wish to seat the instruments in their aligning plates now. Refer to the *Synergy Neo Stacker* or *BioStack's Operator's Manual* for more information.

5: Remove the Shipping Hardware

	Remove all shipping hardware before you turn on the reader.

1. Locate the shipping hardware: carrier shipping bracket and hardware, the top filter chamber shipping brackets, and, if equipped, the bottom filter chamber shipping bracket.





2. Using a #2 Phillips screwdriver, remove the screws holding the carrier shipping bracket. Push the carrier off the rear of the bracket, and then lift and withdraw the bracket from the instrument.
3. Remove the top filter chamber shipping bracket(s).
4. If equipped, remove the bottom filter chamber shipping bracket.
5. Store the shipping brackets and hardware with the original packaging for reuse in case you need to ship the instrument.

The filter cubes are shipped in a protective case. The **Getting Started** chapter explains when and how to install filter cubes.

6: Install the Power Supply

	**Power Rating.** The instrument must be connected to a power receptacle that provides voltage and current within the specified rating for the system. Use of an incompatible power receptacle may produce electrical shock and fire hazards. **Electrical Grounding.** Never use a plug adapter to connect primary power to the instrument. Use of an adapter disconnects the utility ground, creating a severe shock hazard. Always connect the system power cord directly to an appropriate receptacle with a functional ground.

1. Locate the power inlet on the left side of the reader.
2. Examine the power supply's plug. It has three small holes and a small groove that line up with the prongs and a tab inside the power inlet.
3. Insert the plug into the power inlet and finger-tighten the collar of the plug. Plug the power supply's cord into an appropriate power receptacle.

Do **not** plug the power supply into a power receptacle until after the power supply is connected to the instrument.

7: Install the Gas Controller (if applicable)

The gas controller is an external module that enables the user to control CO_2 and O_2 concentrations inside the attached instrument's reading chamber. If you purchased the module for operation with the Synergy Neo2, refer to the *Gas Controller User Guide* for installation instructions.

8: Install the Dispenser

Place the dispense module on top of the reader or on top of the gas controller (if equipped). Do not place the dispenser next to the reader.

Synergy Neo2 with dispenser and with dispenser and gas controller

1. Open the plastic bag containing the injector tube and tips. Remove the clear plastic shrouds from the tubes.
2. Remove the two inlet tubes from their plastic canisters.
3. Identify the two syringe valves on the dispense module. Each is labeled with a left-pointing arrow.

When installing the inlet and outlet tubes, do not use any tools. Finger-tighten only!

4. Screw the fitting of one inlet tube into the right side of the Syringe 1 valve.
5. Identify the #1 outlet tube, and screw it into the left side of the Syringe 1 valve.
6. Repeat these steps to attach the inlet and outlet tubing for Syringe 2.

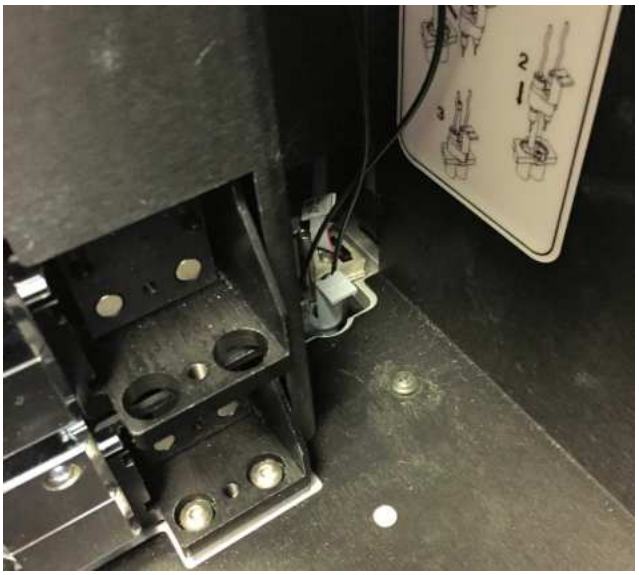
It is critical that the tubing is installed in the correct ports. Otherwise, injected fluid may miss the intended well.

7. Remove the round tubing feed-through cover from the top of the reader (2 screws). Store the cover and screws with the shipping hardware in case the reader needs to be shipped again.
8. Thread the injector tip holder, with outlet tubing connected to both ports, through the hole in the top of the reader.

9. Open the reader's top door, and, holding the injector tip holder by the tab, insert the injector tips into the appropriate holes inside the reader, behind the filter cube chamber(s).

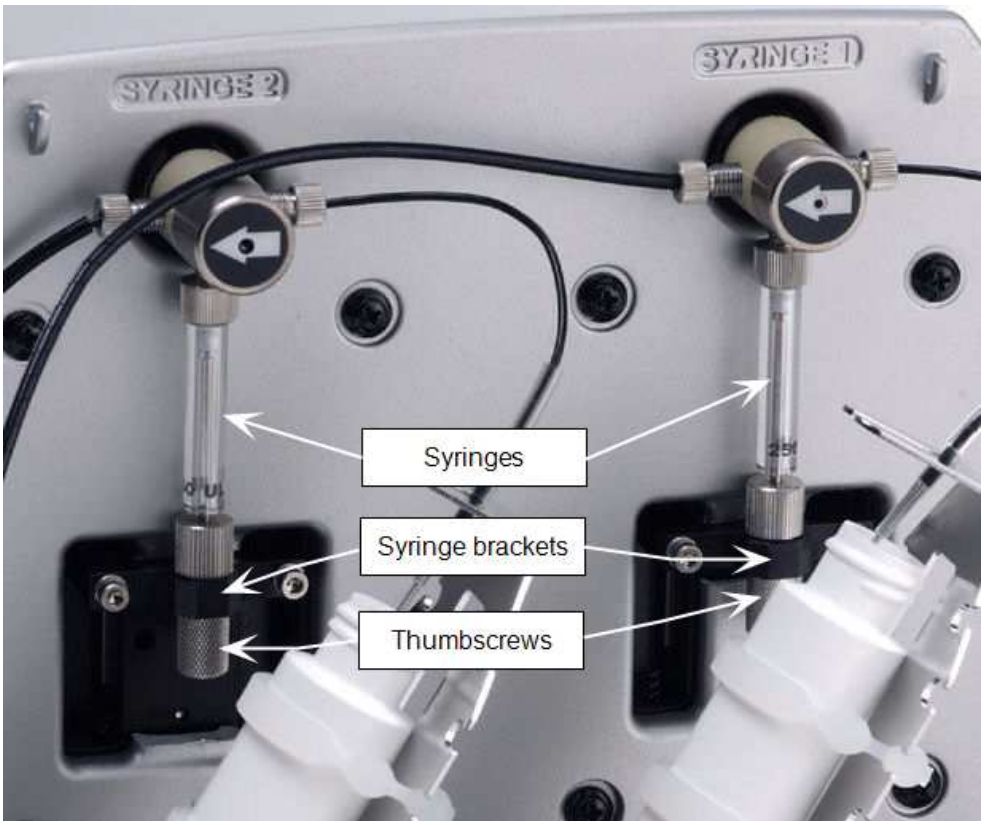
Removing any filter cubes that are installed in the filter cube chambers may be necessary to reach the injector tip holes.

Injector tip holder in its socket



A magnet located between the injector tips helps to guide the tips into place and secures them in the reader.

10. Place the tubing feed-through cover over the hole in the top of the reader, and finger-tighten the thumbscrews to secure it.
11. Remove the two syringes from their protective boxes. They are identical and interchangeable.
12. Install both syringes.
    - Hold the syringe vertically with the threaded end at the top.
    - Screw the top of the syringe into the bottom of the syringe valve. Finger-tighten only.
    - Carefully pull down the bottom of the syringe until it rests inside the hole in the bracket.
    - Pass a thumbscrew up through this hole and thread it into the bottom of the syringe. Hold the syringe to prevent it from rotating while tightening the thumbscrew. Finger-tighten only.



13. Locate the dispenser cable. Plug one end into the port on the back of the dispenser. Plug the other end into the “Dispenser Port” on the left side of the reader.
14. Locate the injector tip-cleaning stylus, packaged in a small cylinder. Attach the cylinder to the back of the dispenser for storage.

Perform a visual inspection or a Performance Qualification test after reconnecting the tubes.

9: Connect the Host Computer

The Synergy Neo2 is equipped with two communication ports: USB and Serial (RS-232). Both ports are located on the left side of the reader.

- A USB cable is included in the accessories box; the RS-232 cable is available as an optional accessory.
- Connect one end to the appropriate port on the reader and the other end to the appropriate port on the host computer.

10: Install Gen5 on the Host Computer

The Synergy Neo2 is controlled by Gen5 software running on a host computer. There is a certain sequence of events that **must** be followed to ensure that the software is properly installed and configured. Please follow the instructions provided in *Gen5 Getting Started Guide* to install the software.

11: Turn on the Reader

1. If Gen5 is open, close it now.
2. The reader’s power switch is located on the lower-right corner of the front panel. Turn the reader on. The reader performs a system test. When the test is completed, the reader extends the microplate carrier.

The carrier eject button, located above the reader’s power switch, can be used to extend/retract the microplate carrier.

When the reader is idle, the light on the carrier eject button is green. When the reader is busy (when running an assay or a self-test) the light is red. If an error occurs, the light is red and blinking.

12: Establish Communication

If using the USB cable, refer to the instructions that shipped with the USB Driver Software on the Gen5 software media to install the necessary drivers.

1. Start Gen5 and log in if prompted. The default System Administrator password is **admin**.
2. In the Task Manger, select **Setup > Go to System Menu**.
3. From the Gen5 main screen, select **System > Instrument Configuration** and click **Add Reader**.
4. Set the Reader Type to **Synergy Neo2**, and click **OK**.
5. Perform one of the following steps:
   - Select **Plug & Play** and click on the Reader Type you are using.

   A Synergy Neo2 must be connected via USB to the computer and turned on to appear in the Available Plug & Play Readers list.

   - Set the Com Port to the computer's COM port to which the reader is connected.

   If using the USB cable, the information can be found via the Windows Control Panel, under Ports in the Hardware/Device Manager area of System Properties (e.g., USB Serial Port (COM5)).
6. Click **Test Communication**. Gen5 attempts to communicate with the reader. If the communication attempt is successful, return to Gen5's main screen.

Communication Errors

If the communication attempt is not successful, try the following:

- Is the reader connected to the power supply and turned on?
- Is the communication cable firmly attached to both the reader and the computer?
- Did you select the correct Reader Type in Gen5?
- Did you install the USB driver software, if applicable?
- Was the reader allowed to fully complete its power-up self-test before initiating communication with Gen5?

If you remain unable to get Gen5 and the reader to communicate with each other, contact BioTek's Technical Assistance Center.

13: Set Dispenser Calibration Values

Applies only to models equipped with injectors.

Before using the external dispense module with the Synergy Neo2, you must set its calibration values in Gen5.

The calibration values for both dispensers (#1 and #2) are printed on labels affixed to the rear of the dispense module. Each label lists six target calibration value (e.g., 200, 80, 40) with their actual measured values (e.g., 199.3, 79.7, 39.9). You will enter the **measured** calibration values into Gen5.

1. If you have not already done so, power on the instrument, and establish communication.
2. In Gen5, go to **System > Instrument Configuration**, select your instrument, and click **View/Modify**.
3. Click **Setup**, and then select the **Dispenser 1 tab**.
4. On the keyboard, press CTRL+SHIFT+M to enter maintenance mode for the Dispenser 1 window.
5. Enter the syringe calibration values from the corresponding label on the rear of the dispenser box (see **IQ-12, Install the Dispenser**).
6. Click **Send Volumes**, and then click **Get Volumes** to verify that the entered values were sent to the instrument.
7. Select the **Dispenser 2 tab**, and repeat steps 4 through 6 for Dispenser 2.

14: Run a System Test

Running a system test will confirm that the reader is set up and running properly, or will provide an error code if a problem is detected.

1. Turn on the incubator:
   - From the Gen5 main screen, select **System > Instrument Control > Synergy Neo2**.
   - Click the **Pre-Heating** tab.
   - Enter a Requested temperature of at least 37°C and click **On**.

Wait until the incubator temperature reaches the set point before continuing. The temperature is displayed on the toolbar in the instrument's icon.

2. From Gen5's main view, select **System > Diagnostics > Run System Test**. If prompted to select a reader, select **Synergy Neo2** and click **OK**.
3. When the test is completed, a dialog requesting additional information appears. Enter the information and click **OK**.

If a message appears that a pending system test is waiting from the initial power-up self-test, view the pending system test and repeat steps 2 and 3.

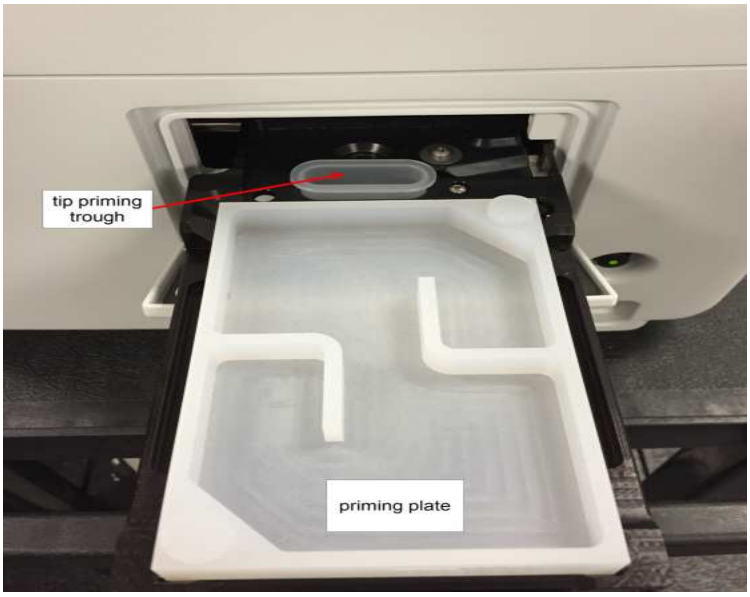
4. The results report appears. Near the top of the report in the Test Results area, the text should read “SYSTEM TEST PASS.”
   - You may wish to print the report and store it with your records. The report can also be saved as a .txt file.
   - The Gen5 software stores system test information in its database; you can retrieve it at any time.
   - Sign and date the report, and store it with your test documentation.

If an error code is returned, refer to **Appendix B** and look up the code. If the problem is something you can fix, do so now and run another system test. If the problem is something you cannot fix, or if the test continues to fail, contact BioTek’s Technical Assistance Center.

5. Turn off the incubator:
   - Select **System > Instrument Control > Synergy Neo2**.
   - Click the **Pre-Heating** tab and click **Off**.

15: Test the Injector System

1. If necessary, press the carrier eject button to extend the microplate carrier.
2. Place the tip priming trough in the rear pocket of the carrier.
3. Place the priming plate on the carrier.



4. Fill the two reagent bottles with distilled or deionized water. Place the bottles in their holders, and place the holders directly in front of the syringes. Insert the inlet tubes into the bottles.
5. From the Gen5 main screen, select **System > Instrument Control > Synergy Neo2**.
6. Click the **Prime** tab.
7. With Dispenser set to **1**, set the Volume to **5000 µL** and click **Prime**.

   The syringe should move down and up repeatedly, drawing fluid from the bottle. The fluid should pump through the tubing and dispense into the priming plate. Examine the fittings; no leaks should be detected. If leaks are detected, tighten all fittings and repeat the prime. If leaks are still detected, contact BioTek's Technical Assistance Center.
8. When the prime finishes, set Volume to **2000 µL** and click **Purge** to clear the fluid lines.
9. Set Dispenser to **2** and repeat steps 7 and 8.
10. When finished, remove and empty the priming plate.
11. Return to the Gen5 main screen.

The installation and setup process is complete.

Operational/Performance Qualification

Your Synergy Neo2 was fully tested at BioTek prior to shipment and should operate properly following the successful completion of the installation and setup procedures described in this chapter.

If you suspect that problems occurred during shipment, if you received the reader back from BioTek following service or repair, or if regulatory requirements dictate that Operational/Performance Qualification is necessary, turn to **Instrument Qualification** now to learn about BioTek's recommended OQ/PQ procedures for Synergy Neo2.

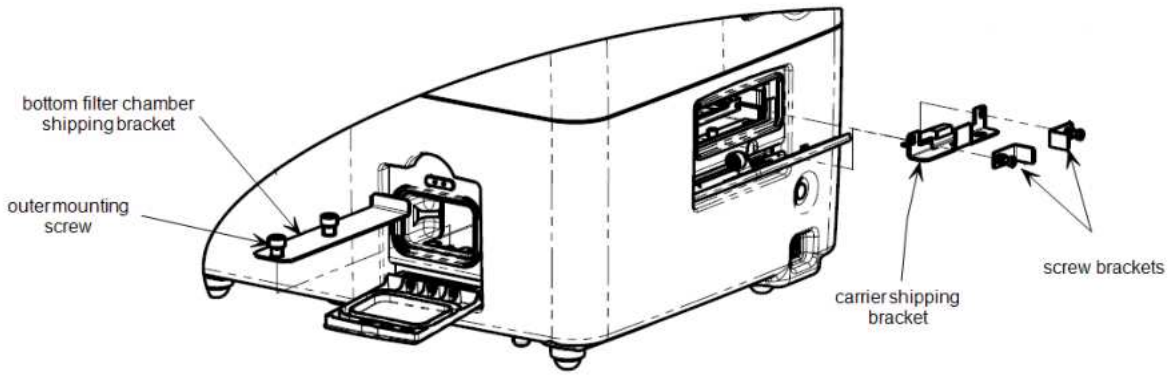
A Product Qualification & Maintenance (IQ/OQ/PQ) package for the Synergy Neo2 is available for purchase (PN 1350526). Contact your local BioTek dealer for more information.

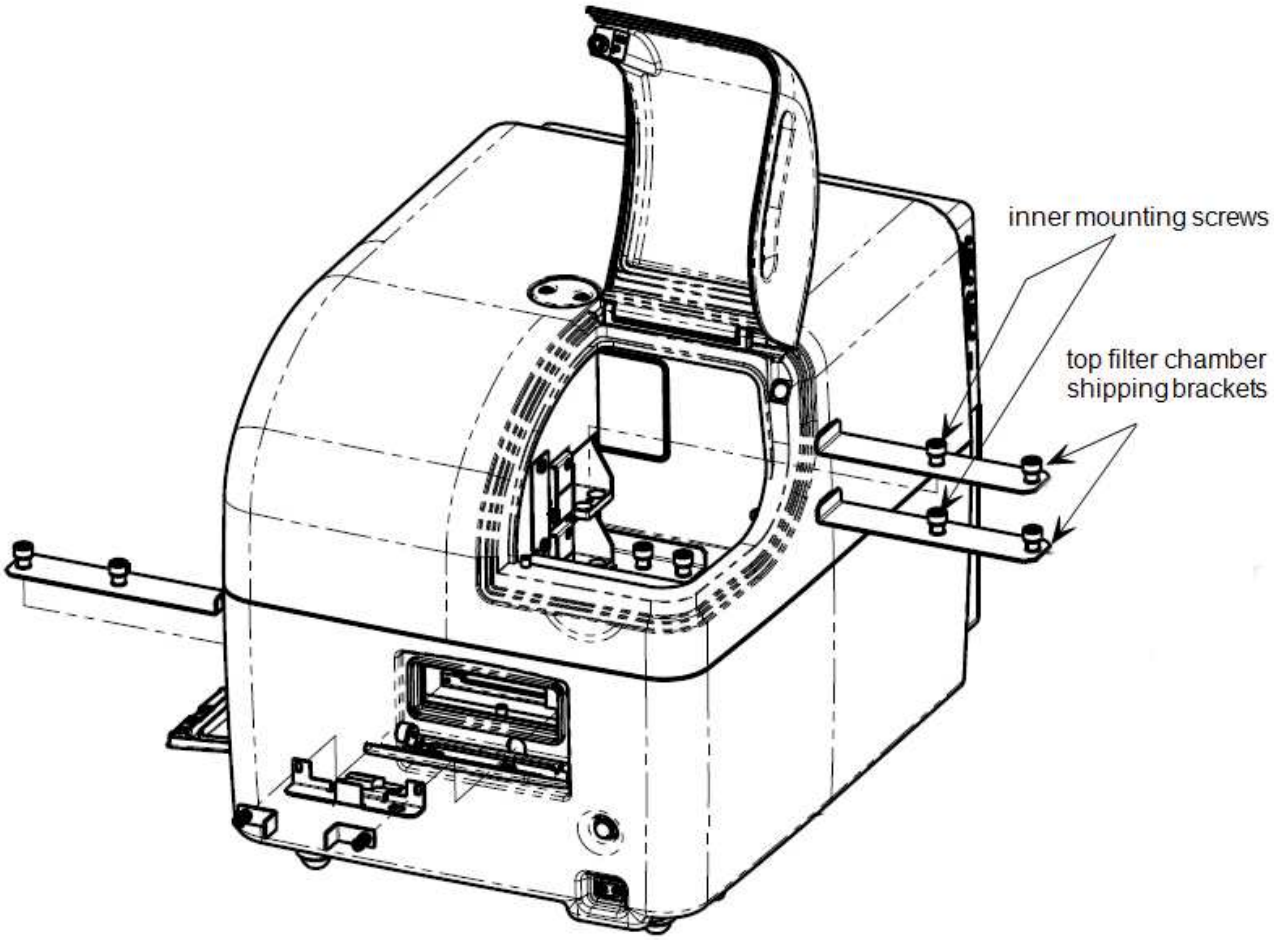
Repackaging and Shipping Instructions

Important! Please read all of the information provided below before preparing the Synergy Neo2 for shipment.

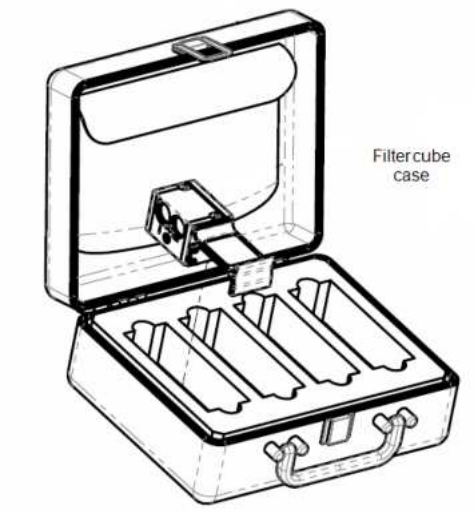
	If the reader and/or dispenser has been exposed to potentially hazardous material, decontaminate it to minimize the risk to all who come in contact with the reader during shipping, handling, and servicing. Decontamination prior to shipping is required by the U.S Department of Transportation regulations. See the **As-Needed Maintenance** chapter for decontamination instructions. Remove the microplate and tip prime trough (if equipped) from the carrier before shipment. Spilled fluids can contaminate the optics and damage the instrument.
	The instrument with all available modules weighs up to **85 lbs. (38.6 kg)**. Use two people when lifting and carrying the instrument.
	The instrument's packaging design is subject to change. If the instructions in this section do not appear to apply to the packaging materials you are using, please contact BioTek's Technical Assistance Center for guidance. Replace the shipping hardware before repackaging the reader. See instructions below. If you have misplaced the shipping hardware, please contact BioTek and order part number 1030009, which includes the following items: • Carrier shipping bracket [x1] (PN 1030539) • Carrier shipping bracket screws [x2] (PN 1032190) • Filter chamber shipping bracket [x3] (PN 1030527) If you need to ship the Synergy Neo2 to BioTek for service or repair, be sure to use the original packaging materials. Other forms of commercially available packaging are not recommended and can void the warranty. You can order replacement shipping materials (PN 1033027) if necessary. The shipping box, accessories box, foam caps, and so on are included as a whole set under this part number and cannot be ordered separately.

Replace the Shipping Hardware





1. Open the carrier door and push the carrier into the reader about 2 inches.
2. Place the main carrier shipping bracket (without the hardware) in the read chamber so that the two holes on the horizontal surface of the bracket slip over the two studs sticking up from the read chamber floor.
3. Pull the carrier back toward the front of the instrument until it rests against the foam piece on the sloped part of the shipping bracket.
4. Holding the carrier against the shipping bracket, place one of the screw brackets against one of the holes at either end of the carrier shipping bracket, so that the screw is pointing toward the hole in the carrier shipping bracket.
5. Insert the screw so that it passes through both shipping brackets and catches the threads in the carrier itself.
6. Repeats steps 4 and 5 to install the second screw bracket.
7. Remove all filter cubes from the instrument. Place them into plastic bags, and then place them into the filter cube case.



8. Install the top filter chamber shipping brackets using the inner mounting screws.
9. If equipped, install the bottom filter chamber shipping bracket using the outer mounting screw.

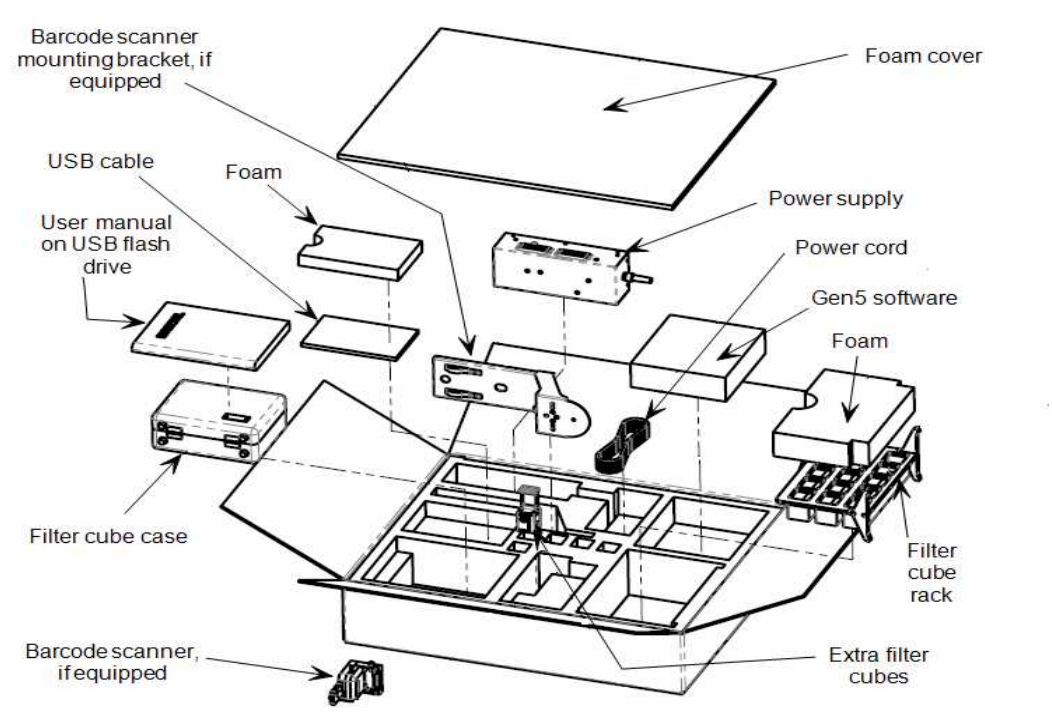
Repack the Instrument

1. Contact BioTek TAC for a Service Call Notice (SCN) number and the shipping address before returning equipment for service.
2. Write the SCN number on the shipping box in large, clear letters.

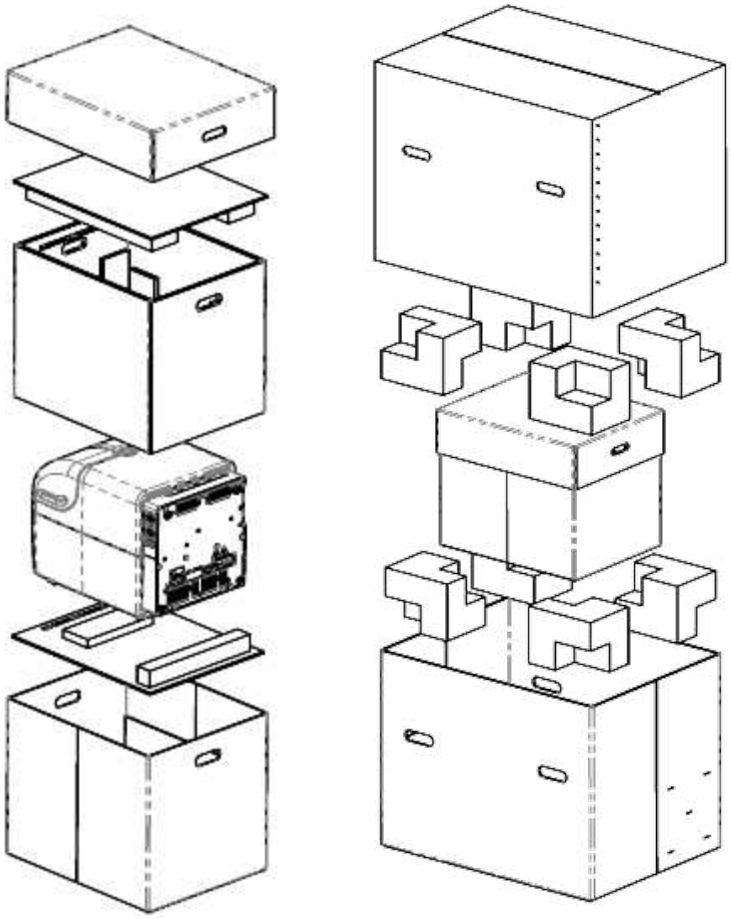
3. Include the SCN number in the shipping address label:
   BioTek Instruments, Inc.
   ATTN: SCN#xxxx
   15 Tigan Street
   Winooski, Vermont 05404 USA
4. Decontaminate the reader and, if attached, the dispense module, according to the instructions provided in the **As-Needed Maintenance** chapter.
5. If you will also be shipping the dispense module, see **Preparing the Dispenser for Shipment** on page 29.

If you are not shipping the dispenser, purge all fluids from the lines, and then disconnect it from the reader.

6. Place the accessories in the accessories box, then seal the accessories box with tape.



7. Place the instrument in the large plastic bag, then place it in the interior box, surrounded by foam boards. Then, place the interior shipping box, surrounded by the foam corners, into the external shipping box. Refer to the next figure.



8. Ship the box to BioTek.

Preparing the Dispenser for Shipment

1. If you have not already done so, contact BioTek's Technical Assistance Center for a Service Call Notice (SCN) number and the shipping address before returning equipment for service.
2. Write the SCN number on the shipping box in large, clear letters.
3. Include the SCN number in the shipping address label:
   BioTek Instruments, Inc.
   ATTN: SCN#xxxxx
   15 Tigan Street
   Winooski, Vermont 05404 USA
4. Decontaminate the dispenser according to the instructions in the **As-Needed Maintenance** chapter. Be sure to purge the dispenser of all fluid when finished.

5. With the reader on, start Gen5 and select **System > Instrument Control > Synergy Neo2**.
6. Perform this step twice, for both dispensers: Click the **Prime** tab and set the dispenser number (1 or 2). Click **Maintenance**. The syringe bracket lowers itself. Remove the thumbscrew from underneath the bracket. Carefully unscrew the top of the syringe from the syringe valve. Lift out the syringe and store it in its original box.
7. Fully detach the dispenser from the reader. (The screws are stored in the plastic bag attached to the back of the dispenser.) Set the dispenser aside for the moment.
8. Remove the tip priming trough and store it in the dispenser accessories bag.
9. Remove the two inlet tubes from the syringe valves and store them in their plastic canisters.
10. Remove the two outlet tubes from the syringe valves. Attach the clear plastic shrouds to the fittings of the outlet tubes. Place the tubes in a plastic bag.
11. Remove the front cover from the dispenser.
12. Insert the bottom foam end cap in the dispenser accessories shipping box and place the accessories in the insert.
13. Insert the bottom foam end cap in the shipping box, and place the dispenser inside the end cap.
14. Insert the foam insert that holds the reagent bottle holders and injector tubing into the shipping box and place the bottle holders and tubing in it.
15. Slide the dispenser accessories box into the shipping box.
16. Insert the top foam end cap. Close and seal the outer box with tape, and ship it to BioTek.

Chapter 3

Getting Started

This chapter describes some of the Synergy Neo2's external and internal components, and provides an introduction to using BioTek Gen5 software to control the instrument.

Modular Design

The Synergy Neo2 is a multi-mode microplate reader, with a design that allows you to initially purchase only the detection capabilities you need and then upgrade later as your requirements expand. Please contact BioTek Customer Care to learn more about your upgrade options.

Gen5 software is used to control the reader. If the reader is connected and turned on, Gen5 will present only those options that apply to your reader model. For example, if your model is not equipped with the alpha detection system, alpha will not be available as a detection method.

The module letters form the part number for each Synergy Neo2 model. This is indicated on a label on the reader.

The configurations in the next table include dual top PMTs for fluorescence and luminescence.

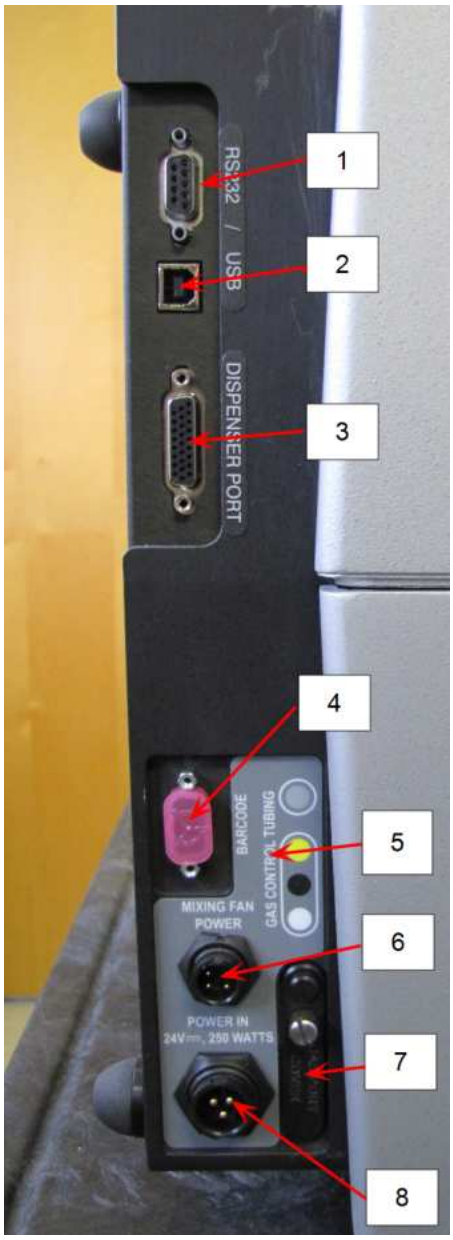
	Monochromator-based		**Filter-based**			**Laser Alpha**
	UV-Vis absorbance	top/bottom FI	FI/FP/TRF	bottom FI/FP/TRF	luminescence	top
NEO2	•		•		•	
NEO2B	•		•	•	•	
NEO2M	•	•	•		•	
NEO2MB	•	•	•	•	•	
NEO2ALPHA	•		•		•	•
NEO2ALPHAB	•		•	•	•	•
NEO2MALPHA	•	•	•		•	•
NEO2MALPHAB	•	•	•	•	•	•

The configurations in the next table include a single top PMT for fluorescence and luminescence.

	Monochromator-based		Filter-based			Laser Alpha
	UV-Vis absorbance	top/bottom FI	FI/FP/TRF	bottom FI/FP/TRF	luminescence	top
NEO2S	•		•		•	
NEO2SB	•		•	•	•	
NEO2SM	•	•	•		•	
NEO2SMB	•	•	•	•	•	
NEO2SALPHA	•		•		•	•
NEO2SALPHAB	•		•	•	•	•
NEO2SMALPHA	•	•	•		•	•
NEO SMALPHAB	•	•	•	•	•	•

External Components

1	Upper and lower top filter cubes access door
2	Light-blocking microplate carrier access door
3	Microplate carrier eject button
4	Power switch
5	Bottom filter cube access door (if equipped)
6	Entry port for the dispense outlet tubes and injectors (if equipped)



1: RS232 cable port

2: USB port

3: Dispenser port

4: Barcode reader cable port (with cover installed)

5: Key for gas controller ports

6: Mixing fan power port

7: Gas controller ports (with cover installed)

8: Instrument power inlet

Internal Components

Component	Description	Page
Barcoded Filter Cubes	The barcoded filter cubes contain excitation and emission filters, mirrors, and polarizing filters. Preconfigured cubes are available from BioTek.	page 36
Injector System	**Applies to models with the Dispense module.** The syringes and tubing may require replacement over time. The tubing and injectors require cleaning at regular intervals.	page 44
Shaking System	The instrument supports six different shake rates from each of three different shake types: linear, slow orbital, and fast orbital.	page 46

Filter Cubes

The Synergy Neo2 is equipped to use up to three filter cubes to perform filter-based fluorescence, luminescence, and alpha reads. Each cube consists of excitation and emission filters, dichroic mirrors, polarizing filters, and/or unique apertures. When placed into the instrument, the unique component information for each cube is automatically identified via an internal barcode scanner.

If two or more custom filter cubes (with ID 255) are configured in Gen5, it is the customer's responsibility to install the correct filter cube when performing a read.

The top filter cubes are accessed through the hinged door on the front of the reader. The bottom filter cube (if equipped) is accessed through a door on the left side of the reader. See the photo under **External Components** on page 34.

Each filter cube is labeled with an ID number (e.g., "107" or "61") and a short description of its contents and purpose (e.g., "360/460, 485/528" or "FP 485/530, LUM"). Each cube also has a barcode label that represents this information.

Be careful not to scratch the surface of the barcode label (see below). If the label is damaged, the Synergy Neo2 may have trouble reading it correctly, requiring you to manually enter the filter cube information in Gen5.

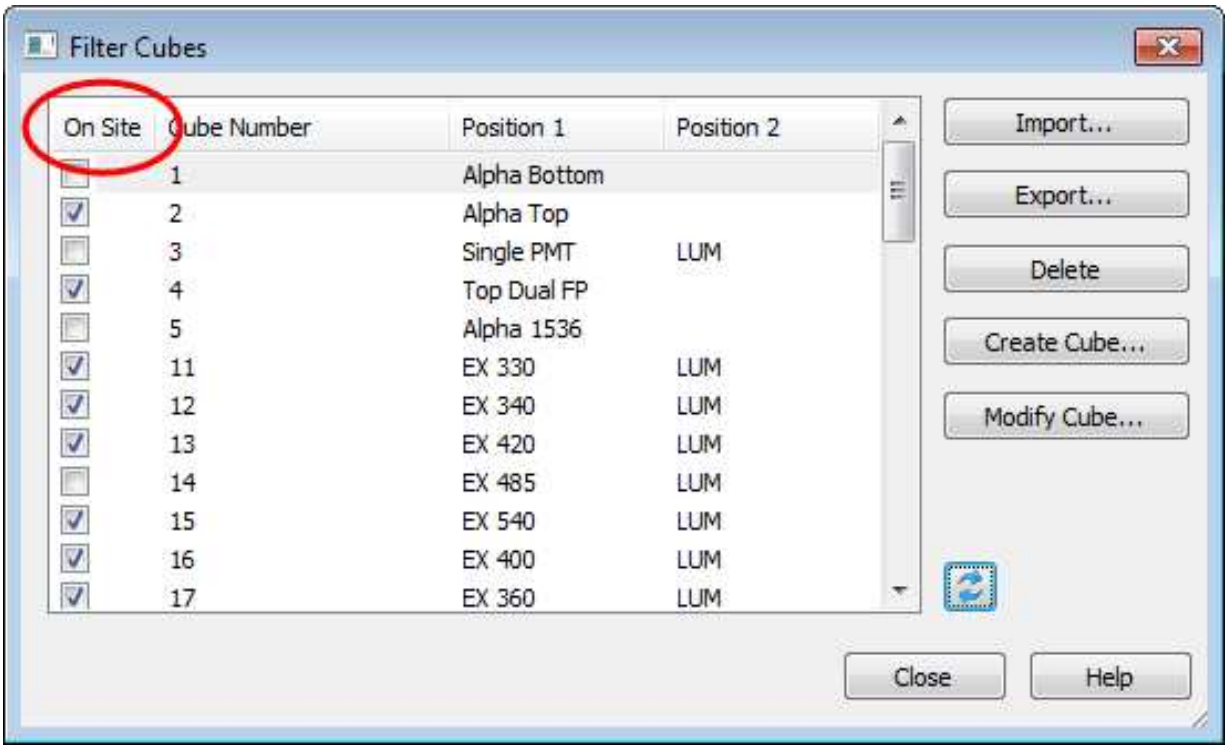
Filter Cubes and Gen5

Refer to the Gen5 Help system for more detailed information and instructions.

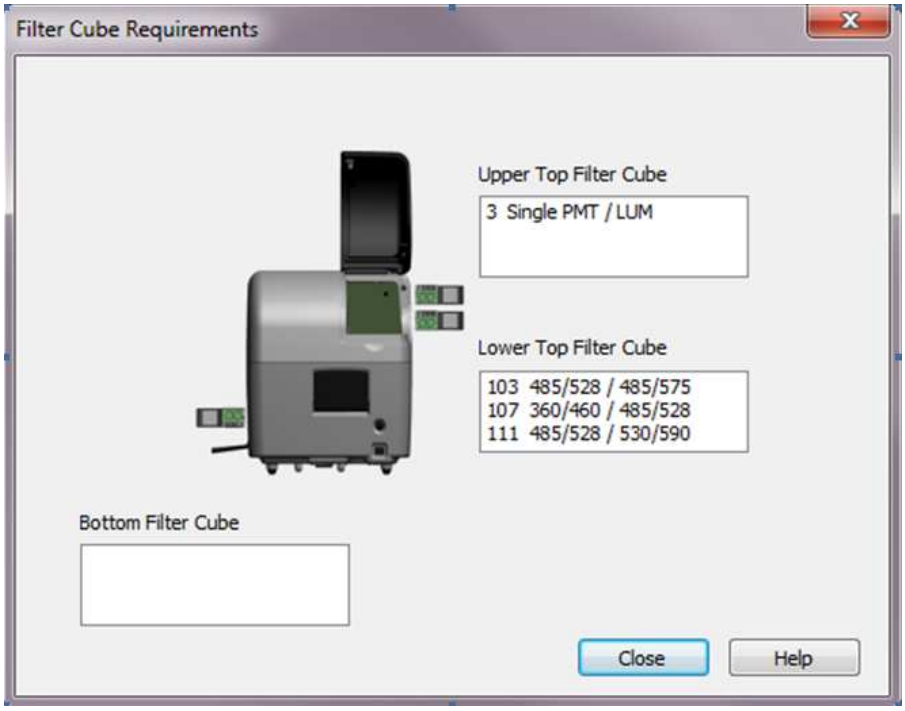
When the Synergy Neo2 reads a new filter cube barcode label, information such as cube position type, filter and mirror specifications, and PMT location is passed on to Gen5.

You can view the filter cube's ID number by selecting **System > Information Configuration > Synergy Neo2 > View/Modify > Setup**. If a location shows "?", either no filter cube is installed or the system could not read the barcode label. You can manually enter the filter cube ID number using the Override feature.

You can use the Gen5 Filter Cube Library (**System > Optics Library > Filter Cubes**) to define all cubes that you have available at your site and to import filter cube definitions, if necessary. Read step options in a protocol or experiment will be available for selection based on the filter cubes that you identify as being "On Site."



Gen5 also uses the filter cube information to prompt for filter cube installation at runtime, if necessary.



Installing or Removing a Filter Cube

Filter cubes can be installed or removed when the instrument is turned on or off, with one exception:

> Do not open the filter cube access doors while a plate is being read (unless Gen5 prompts you to install a particular filter cube). Doing so may affect measurements and result in invalid data.

Open the top or side access door, as appropriate. Gently slide the filter cube into (or out of) its chamber, and then close the door. The barcode label will be scanned upon door closure.

Cleaning Filter Cubes

> Do not disassemble filter cubes.

Refer to **Inspect/Clean Filter Cubes** in **Preventive Maintenance**.

Filter Cubes Available from BioTek

Preconfigured barcoded filter cubes are available for purchase from BioTek; see the list at www.biotek.com/products/accessories. Please note that part numbers are subject to change, and new filter cubes may become available. Custom filter cubes are also available. Contact BioTek Customer Care with any questions.

Custom Filter Cubes

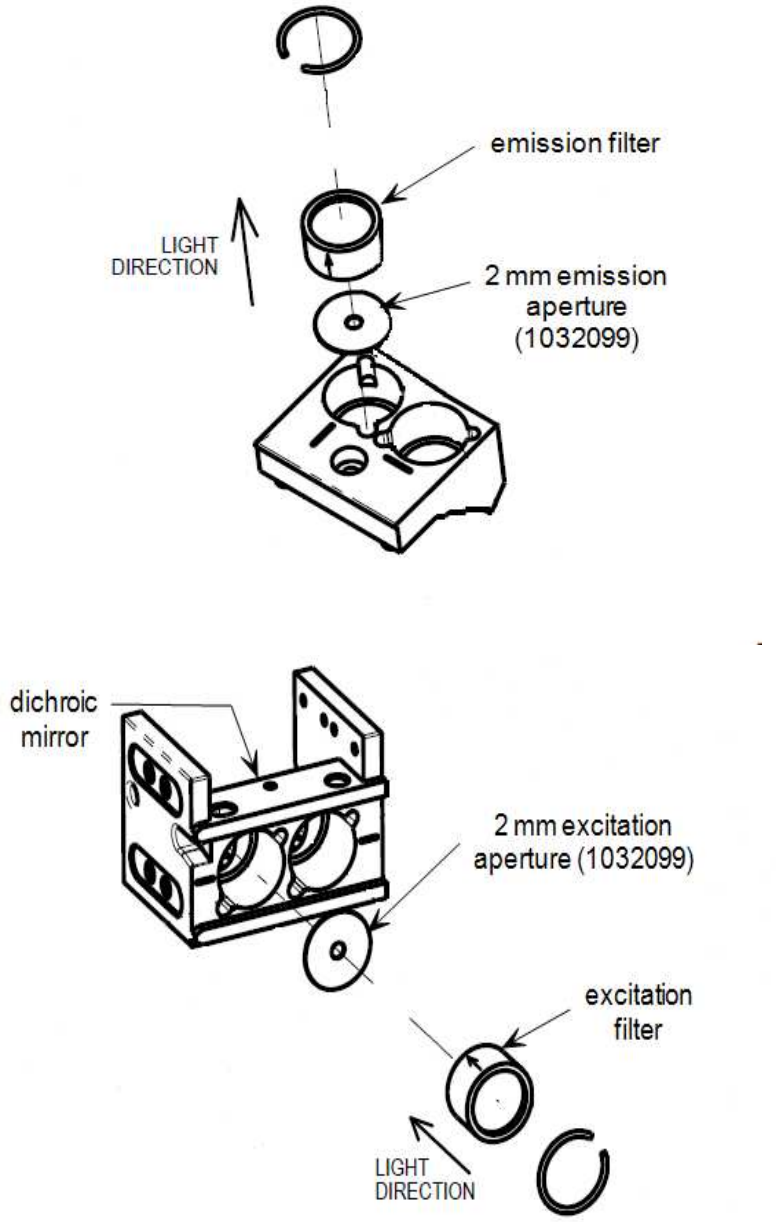
> Custom barcoded filter cubes are assembled at BioTek. The information in this section is for reference purposes.

> Custom barcoded filter cubes support single PMT measurements. For dual simultaneous-measurement filter cubes (e.g., FP, TR-FRET), please contact BioTek.

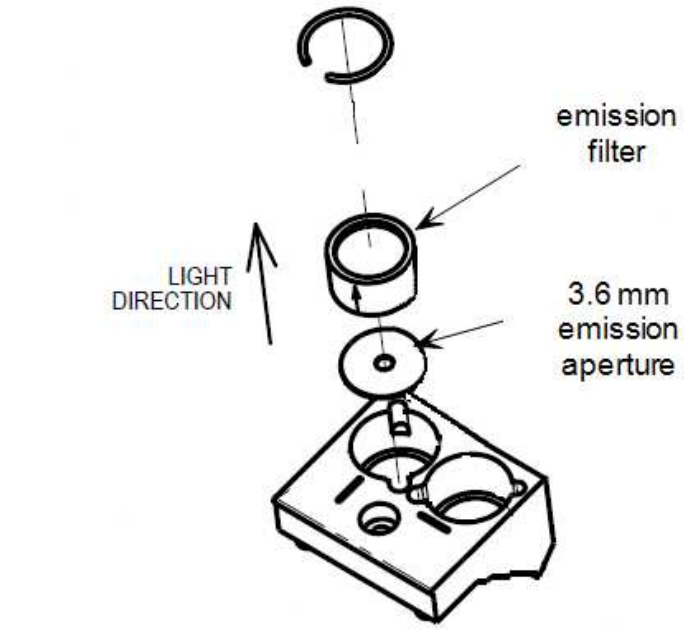
The Synergy Neo2 supports the use of custom filter cubes. Each custom cube has the same ID: 255. It is the user's responsibility to ensure that the correct custom filter cube is installed before performing a read.

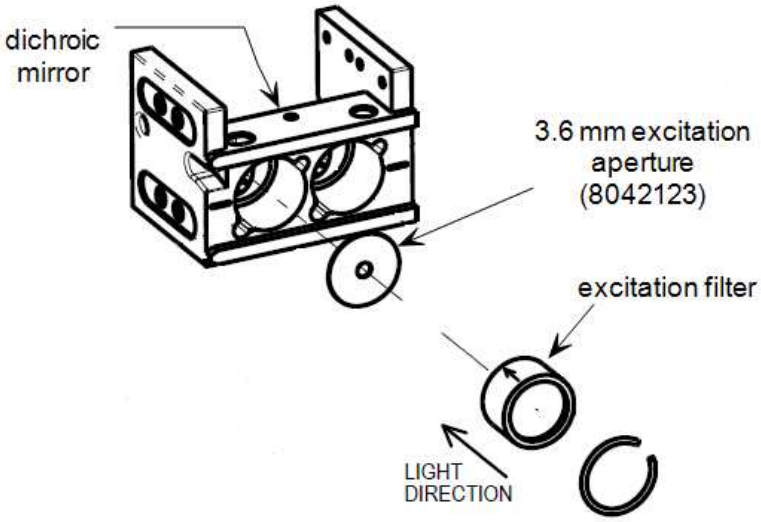
The following drawings shows the orientation of the filters inside the cubes.

Fluorescence Intensity Filter Cube: Use Position 1 or 2

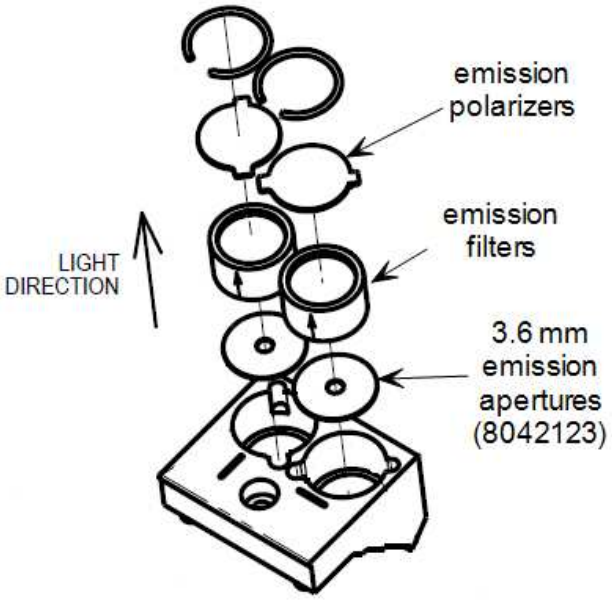


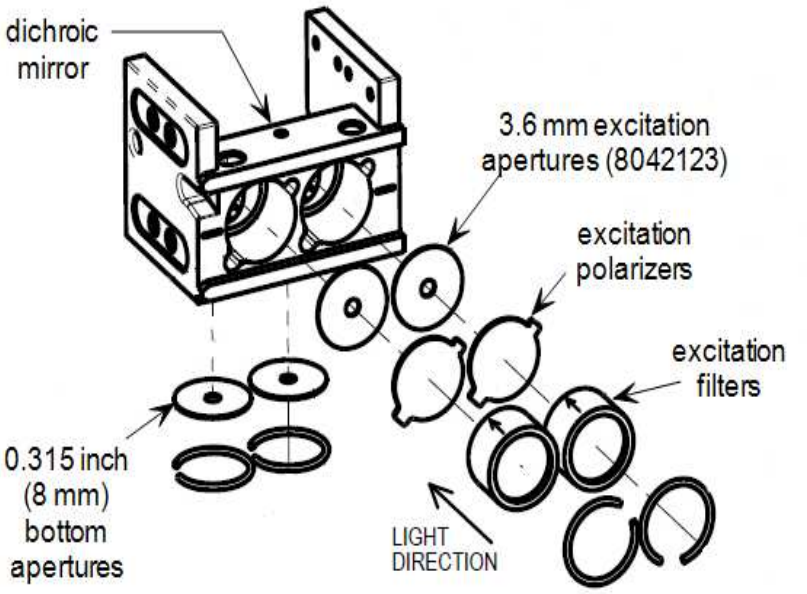
TRF Filter Cube: Use Position 1 or 2



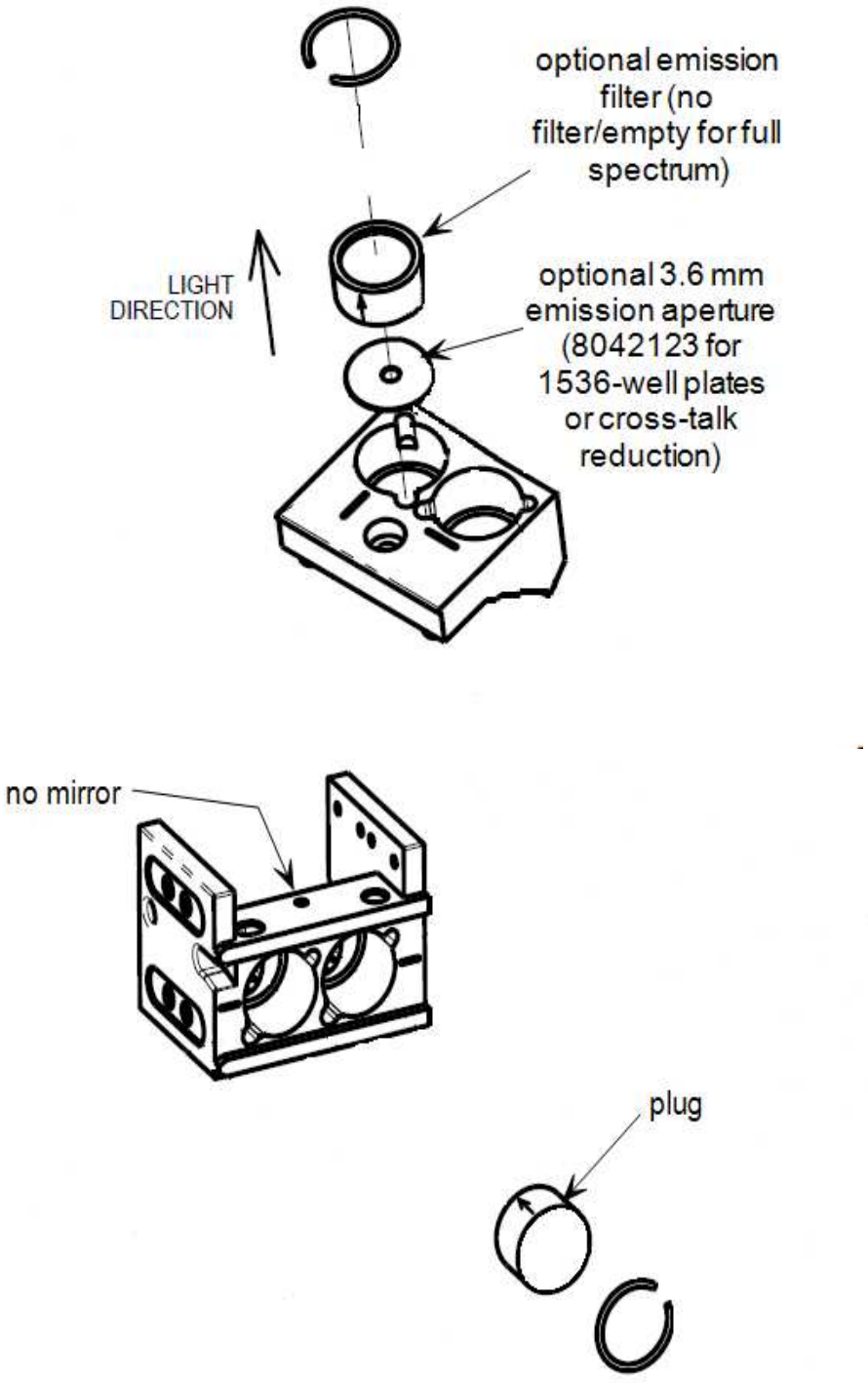


FP Filter Cube: Use both Positions 1 and 2





Luminescence Filter Cube: Use Position 1 or 2



Creating or Modifying Custom Barcoded Filter Cubes in Gen5

Each custom filter cube ordered from BioTek has the same barcode ID: "255." You must enter the custom filter cube's information in Gen5.

> If you define two or more filter cubes with ID 255, it is your responsibility to install the correct filter cube when performing a read. To facilitate this, it is recommended to match the **Cube name** field in Gen5 with the name you write on the cube's label.

To create a new cube:

1. Click **System > Optics Library > Filter Cubes > Create Cube**.
2. Enter a unique Cube Name.
3. Select the detection type for Position 1 (away from the cube's handle), and then click **Edit**.
4. Define information as applicable to the selected detection type. Click **Help** for assistance. Click **OK** with finished.
5. Repeat steps 3 and 4 for Position 2 (closest to the cube's handle), if used.

To modify an existing cube:

1. Click **System > Optics Library > Filter Cubes**.
2. Select the desired cube and click **Modify Cube**.
3. Edit information as necessary.

Injector System

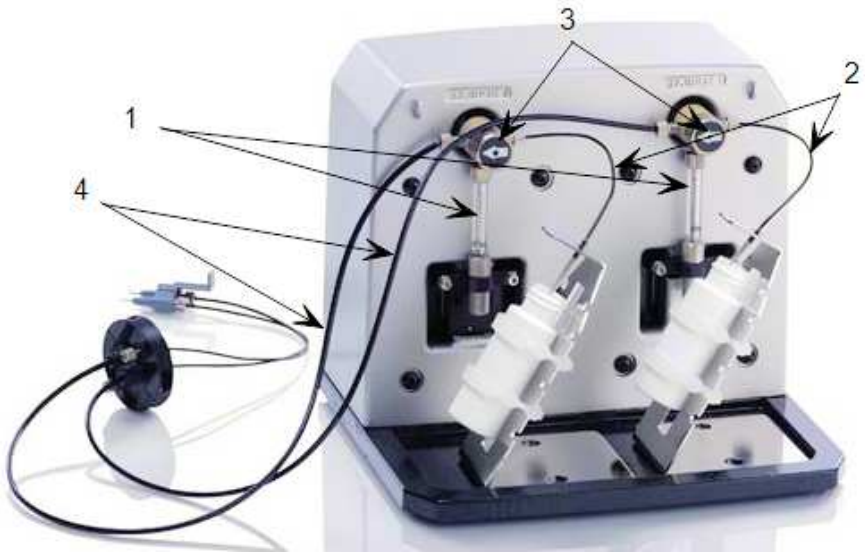
> The tubing and injectors should be cleaned at least every three months. See **Preventive Maintenance**, for instructions.

> Inspect the injector system daily for leaks, preferably immediately after priming and whenever tubing changes have been made.

> If a syringe is leaking, it may need to be replaced. See **As Needed Maintenance**, for instructions.

Dispense Module

The dispense module sits on top of the reader and pumps fluid from the reagent bottles to the injectors located inside the instrument. Fluid is injected into one well at a time. The injectors support plate types from 6- to 384-wells.



1 Two 250 µL syringes draw fluid from the supply bottles.

2 Inlet tubes transport fluid from the supply bottles to the syringes. These tubes are short pieces of opaque PTFE (Teflon) tubing connected to stainless-steel probes on one end and threaded fittings on the other end.

3 Solenoid valves allow the fluid drawn from the supply bottles by the syringe pumps to flow into the outlet tubes.

4 Outlet tubes transport fluid from the syringes into the instrument, through the tubing ports on the Synergy Neo2's top cover. The outlet tubes are opaque PTFE tubes with threaded fittings on each end.

	Avoid continuous contact with harsh chemicals. Rinse the fluid path with deionized water after contact with any strong acid, base, or solvent. For information on the materials used in the injection system, refer to *Injection System—Chemical Compatibility Technical Note* on the USB flash drive supplied with the Synergy Neo2.

See the **Preventive Maintenance** chapter for cleaning instructions.

Priming the Injector System

Before running a dispense assay, prime the system with the reagent or dispensing fluid. In addition, tip priming can be performed at the start of an assay and, sometimes, just before each dispense to a well. The tip prime compensates for any fluid loss at the injector tip due to evaporation since the last dispense. All priming activities are controlled via Gen5.

If the injector system is not primed adequately, air bubbles can get trapped in the system and affect injection volumes. Air bubbles in the system can also result in fluid spraying or scattering inside the reader.

Both types of primes require a fluid reservoir to be present on the microplate carrier.

- The priming plate is placed on the microplate carrier for a Prime operation (to prime the dispense system with fluid).
- The tip priming trough is placed in the rear pocket of the carrier, and is used for performing the Tip Prime before dispensing. The trough holds up to 1.5 mL of liquid and must be periodically emptied and cleaned by the user.

Do not perform tip priming when using tall plates. Generally, plates with fewer than 96 wells are too tall for error-free tip priming; and, tip priming is rarely required for these larger-volume plates.

The priming tray should be empty before priming and should contain fluid after priming.

Shaking System

The user can choose six different shake rates from each of three different shake types: linear, slow orbital, and fast orbital. Each shake rate corresponds to a specific carrier displacement for one or both axes, ranging from 1 mm to 6 mm. A linear shake simply moves the carrier's y-axis back and forth in a line, whereas an orbital shake moves both carrier axes to scribe a circle. Each orbital type may also be selected in a double format, in which the carrier scribes a figure-eight pattern instead.

Carrier Shake Definitions

Shake Type	Displacement (mm/steps)	Period (msecs)	Frequency (Hz)	RPM	Ramp Profile
Linear	1/6	54.8	18.3	1096	13
	2/12	82.0	12.2	731	14
	3/18	105.8	9.5	567	15
	4/24	121.6	8.2	493	16
	5/30	146.3	6.8	410	17
	6/36	166.9	6.0	360	18
Slow orbital	1/6	107.3	9.3	559	19
	2/12	164.4	6.1	365	20
	3/18	212.5	4.7	282	21
	4/24	253.5	3.9	237	22
	5/30	292.1	3.4	205	23
	6/36	334.2	3.0	180	24
Fast orbital	1/6	74.3	13.4	807	25
	2/12	109.6	9.1	548	26
	3/18	141.3	7.1	425	27
	4/24	169.0	5.9	355	28
	5/30	195.4	5.1	307	29
	6/36	222.8	4.5	269	30

Each shake consists of a series of repeated ramped moves for one or both carrier axes. The sinusoidally defined ramps are used for acceleration and deceleration only; the plateau section of the ramp is skipped. When an axis reaches the end of the ramp profile, it simply repeats the ramp in the opposite direction (except for double-orbital shakes, where the y-axis repeats a ramp twice before changing directions).

The circular pattern of the orbital shake is achieved by starting the carrier's y-axis move sequence first. When that axis reaches peak speed at the top of its ramp, the carrier's x-axis begins its own series of repeated moves using the same profile. The axes remain in synch

but out of phase, with the maximum speed of one always coinciding with the minimum speed of the other, until the specified shake time has expired.

Maximum Shaking Amplitude Based on Assay Volume and Plate Type

The Synergy Neo2 offers a broad range of shaking speeds and amplitudes. If the wells of a microplate are almost full, shaking can result in spillage inside the instrument. The following table is designed to help avoid this issue. Select your microplate type and assay volume, and you will get the acceptable shake amplitude on your instrument.

Sample Volume	Linear	Orbital Slow	Orbital Fast	Double Orbital Slow	Double Orbital Fast
6-well Microplate					
0–3 mL	1–6 mm	1–6 mm	1–6 mm	1–6 mm	1–6 mm
3–4 mL	1–6 mm	1–6 mm	1–6 mm	1–6 mm	1 mm
4–5 mL	1 mm	1–6 mm	1–6 mm	1–6 mm	1 mm
5–7 mL	1 mm	1–6 mm	1–3 mm	1 mm	No
7–8 mL	1 mm	No	1–3 mm	No	No
9 mL	1 mm	No	No	No	No

Sample Volume	Linear	Orbital Slow	Orbital Fast	Double Orbital Slow	Double Orbital Fast
12-well Microplate					
0–1.5 mL	1–6 mm	1–6 mm	1–6 mm	1–6 mm	1–6 mm
1.5–2.5 mL	1–2 mm	1–6 mm	1–6 mm	1–6 mm	1 mm
2.5–3 mL	1–2 mm	1–6 mm	No	No	1 mm
3–4 mL	No	1–6 mm	No	No	No

Sample Volume	Linear	Orbital Slow	Orbital Fast	Double Orbital Slow	Double Orbital Fast
24-well Microplate					
0–0.75 mL	1–6 mm	1–6 mm	1–6 mm	1–6 mm	1–6 mm
0.75–1 mL	1 mm	1–6 mm	1–6 mm	1–6 mm	1–6 mm
1–1.5 mL	1 mm	1–6 mm	1–6 mm	1–6 mm	No
1.5–2 mL	1 mm	1–6 mm	No	1–6 mm	No

Sample Volume	Linear	Orbital Slow	Orbital Fast	Double Orbital Slow	Double Orbital Fast
48-well Microplate					
0–0.5 mL	1–6 mm	1–6 mm	1–6 mm	1–6 mm	1–6 mm
0.5–1 mL	1 mm	1–6 mm	1–6 mm	1–6 mm	1–6 mm
1–1.3 mL	No	1–6 mm	1–6 mm	1–6 mm	No

Sample Volume	Linear	Orbital Slow	Orbital Fast	Double Orbital Slow	Double Orbital Fast
96-well Microplate					
0–0.25 mL	1–6 mm	1–6 mm	1–6 mm	1–6 mm	1–6 mm

Gen5 Software

BioTek Gen5 software supports all Synergy Neo2 reader models. Use Gen5 to control the reader, the dispense module (if equipped), and the stacker (if equipped); perform data reduction and analysis on the measurement values; print or export results; and more. This section provides brief instructions for working with Gen5 to create protocols and experiments and read plates. Refer to the Gen5 Help system for more information.

Protocols and Experiments

In Gen5, a protocol contains instructions for controlling the reader and (optionally) instructions for analyzing and reporting or exporting the data retrieved from the reader. At

a minimum, a protocol must specify the procedure for the assay you wish to run. After creating a protocol, create an experiment that references the protocol. You'll run the experiment to read plates and analyze the data.

These instructions briefly describe how to create a protocol in Gen5. See the Gen5 Help system for complete instructions.

1. Create a new protocol.
2. Open the Procedure dialog. If prompted to select a reader, select the **Synergy Neo2** and click **OK**.
3. Select a Plate Type.

The Plate Type selected in Gen5 must match the assay plate. Otherwise, the results of the read may be invalid. Also, a collision may result with the transport system because of differing plate dimensions. Be sure to select the **Use lid** check box, if applicable.

4. Add steps to the procedure for shaking or heating the plate, dispensing fluid, reading the plate, and more. Click **Validate** to verify that the reader supports the defined steps, and then click **OK**.

Optionally, perform the next steps to analyze and report the results:

5. Open the Plate Layout dialog and assign blanks, samples, controls, and/or standards to the plate.
6. Open the Data Reduction dialog to add data reduction steps. Categories include Transformation, Well Analysis, Curve Analysis, and Qualitative Analysis/QC.
7. Create a report or export template via the Report/Export Builders.
8. Select **File > Save** and give the file an identifying name.

These instructions briefly describe how to create an experiment and then read a plate in Gen5. See the Gen5 Help system for complete instructions.

1. Create a new experiment using an existing protocol.
2. Select the desired protocol and click **OK**.
3. Select a plate in the menu tree and read it.
4. When the read is complete, measurement values appear in Gen5. Select the desired data set from the Data list.
5. Select **File > Save** and give the file an identifying name.

Dispense Module Control

This section applies only to models with injectors.

Gen5 is used to perform several dispense functions, such as initialize, dispense, prime, and purge. The Prime and Purge functions are introduced here. See the Gen5 Help system for more information.

Priming routines are used to fill the dispense tubing with fluid so that air is not dispensed first, resulting in an incorrect dispense volume. The purging routines are used to clean the fluid paths. See also **Flushing/Purging the Fluid Path** in the **Preventive Maintenance** chapter.

Prime

Before running an experiment with a dispense step, prime the system with the fluid to be used.

1. Place the priming plate on the carrier.
2. Fill the supply bottle with a sufficient volume of the fluid to be used for the prime and the assay. Insert the appropriate inlet tube into the bottle.
3. In Gen5, select **System > Instrument Control > Synergy Neo2** and click the **Prime** tab.
4. Select the Dispenser number (1 or 2) associated with the supply bottle.
5. Enter the Volume to be used for the prime. The minimum recommended prime volume is 2000 µL.
6. Select a prime Rate, in µL/second.
7. Click **Prime** to start the process.
8. When finished, carefully remove the priming plate from the carrier and empty it.

If the priming plate is empty, the prime volume was too low or there is a problem with the dispense system.

Purge

To save reagent, Gen5 provides the option to purge fluid from the dispense tubing back into the supply bottle.

1. In Gen5, select **System > Instrument Control > Synergy Neo2** and click the **Prime** tab.

2. Select the Dispenser number (1 or 2) associated with the supply bottle.
3. Enter the desired purge Volume in µL (e.g., 2000).
4. Select a prime Rate in µL/second.
5. Click **Purge** to start the process.

Recommendations for Optimum Performance

General

- Microplates should be clean and free from dust or bottom scratches. Use new microplates from sealed packages. Do not allow dust to settle on the surface of the solution; use microplate covers or seals when not reading the plate. If reading plates with covers still installed, do not forget to compensate for this. See the Gen5 Help.
- Filter solutions to remove particulates that could cause erroneous readings.
- Although the Synergy Neo2 supports standard flat, U-bottom, and V-bottom microplates, the reader achieves optimum performance with flat-bottomed wells when running in Absorbance mode. See **Appendix A, Specifications** for more information on the supported plates.
- Non-uniformity in the optical density of the well bottoms can cause loss of accuracy, especially with U- and V-bottom polyvinyl microplates. Check for this by reading an empty microplate. Dual wavelength readings can eliminate this problem, or bring the variation in density readings to within acceptable limits for most measurements.
- Inaccuracy in pipetting has a large effect on measurements, especially if smaller volumes of liquid are used. For best results in most cases, use at least 100 µL per well in a 96-well plate and 25 µL in a 384-well plate.
- Pipetting solution into 384-well plates often traps air bubbles in the wells, which may result in inaccurate readings. A dual-wavelength reading method usually eliminates these inaccuracies. For best results, however, remove the air bubbles by degassing the plate in a vacuum chamber or spinning the plate in a centrifuge before reading.
- The inclination of the meniscus can cause loss of accuracy in some solutions, especially with small volumes. Shake the microplate before reading to help bring it within acceptable limits. Use Tween 20, if possible (or some other wetting agent) to normalize the meniscus for absorbance measurements. Some solutions develop menisci over a period of several minutes. This effect varies with the brand of microplate and the solution composition. As the center of the meniscus drops and

shortens the light path, the density readings change. The meniscus shape will stabilize over time.

- It is the user's responsibility to understand the volumetric limits of the plate type in use as it applies to the assay being run.
- Use UV transparent microplates for UV wavelength reads.
- Use of liquids with concentrations of acids, corrosives, or solvents of 3 percent and greater can begin attacking the materials inside the instrument's chamber. Running multiple plates with concentrations < 3 percent in long kinetics may also have a destructive effect. If the experiment is incubated, it will accelerate the deterioration of chamber components. When in doubt about the use of acids, corrosives, or solvents, please contact TAC@biotek.com.

Read Direction

The Synergy Neo2 performs most reads in a column-wise direction,that is, moving from well A1, to B1, then C1, and so on. The exception to this is for reads that use sweep speed and random well reads, which are read in a row-wise fashion.

Luminescence Measurements

For highly sensitive luminescence assays using white plates, add a Delay step to your procedure to "dark adapt" the plates in the Synergy Neo2's reading chamber before taking measurements.

Monochromator-Based Fluorescence Systems

Although Time-Resolved Fluorescence can be performed with the monochromator, the filter-based fluorescence system is more sensitive for TRF and is the better choice.

Models with Injectors

- To keep the dispense system in top condition, flush and purge the fluid lines with deionized (DI) water every day or upon completion of an assay run, whichever is more frequent. Some reagents may crystallize or harden after use, clogging the fluid passageways. Flushing the tubing at the end of each day, letting the DI water soak, and then purging the lines at the beginning of each day ensures optimal performance of the dispense system. See the **Preventive Maintenance** chapter for more information.
- When dispensing volumes less than or equal to 20 µL/well, we recommend specifying a tip prime volume that is equal to the dispense volume. For dispense volumes greater than 20 µL/well, we recommend a tip prime volume of 20 µL.
- To avoid spillage and possible contamination of the instrument, empty the tip prime trough frequently and do not exceed the total fluid volume of the plate well when dispensing.

Using 384-Well Microplates

When using a 384-well microplate, you can use the Gen5 Auto Map feature to ensure you are using an accurate plate map for your reads. See the Gen5 Help for more information.

Incubation and Partial Plates

When performing a partial plate read that includes an incubation step, the following recommendations can reduce the effects of evaporation of your samples:

- Use microplate lids.
- Fill unused wells with fluid.
- Cluster your sample wells rather than spacing them throughout the plate.
- Place your sample wells in the center of the plate. This placement may lead to less evaporation than if you place the samples in wells on the edge of the plate.

Alpha Laser Detection

When using alpha detection, ensure that the relative humidity remains below 85% and the ambient temperature is less than 30°C.

Chapter 4

Preventive Maintenance

This chapter provides instructions for maintaining the Synergy Neo2 and external dispense module (if used) in top condition, to ensure that they continue to perform to specification.

Preventive Maintenance Overview

A general preventive maintenance regimen for all Synergy Neo2 models includes periodically cleaning all exposed surfaces and inspecting/cleaning the filter cubes.

For models with the external dispense module, additional tasks include flushing/purging the fluid path and cleaning the tip prime trough, priming plate, supply bottles, dispense tubing, and injectors.

Daily Cleaning for the Dispense Module

To ensure accurate performance and a long life for the dispense module and injectors, flush and purge the fluid lines with deionized (DI) water every day or after completing an assay run, whichever is more frequent. Some reagents may crystallize or harden after use and clog the fluid passageways. Take special care when using molecules that are active at very low concentrations (e.g., enzymes, inhibitors). Remove any residual reagent in the dispense lines using a suitable cleaning solution (review the reagent's package insert for specific recommendations).

Flushing the tubing at the end of each day, letting the DI water soak overnight, and then purging the lines at the beginning of each day ensures optimal performance of the dispense system. BioTek recommends performing a visual inspection of the dispense accuracy before running an assay protocol that includes dispense steps.

BioTek also recommends flushing the module with DI water before conducting the decontamination procedure described in the **As Needed Maintenance** chapter.

Models with injectors: Accumulated algae, fungi, or mold may require decontamination. See the **As-Needed Maintenance** chapter for complete decontamination instructions.

Preventive Maintenance Schedule

The risk and performance factors associated with your assays may require performing some of all of the procedures more frequently than presented in this schedule.

Task	Page	Daily	Quarterly	As Needed
All models:				
Clean exposed surfaces	page 58			x
Inspect/clean filter cubes	page 58		x	
Decontamination	see note below	before shipment or storage		
Models with injectors:				
Flush/purge the fluid path	page 59	x		
(Optional) Run Dispense protocol	page 60			x
Empty/clean tip prime trough	page 61	x		
Clean priming plate	page 62			x
Clean dispense tubes and injectors	page 62		x	x

Find decontamination instructions in the **As-Needed Maintenance** chapter.

Warnings and Precautions

Read the following before performing any maintenance procedures:

	**Warning! Internal Voltage.** Turn off and unplug the instrument for all maintenance and repair operations.
	**Important!** Do not immerse the instrument, spray it with liquid, or use a dripping-wet cloth on it. Do not allow water or other cleaning solution to run into the interior of the instrument. If this happens, contact BioTek's Technical Assistance Center.
	**Important!** Do not apply lubricants to the microplate carrier or carrier track. Lubricant attracts dust and other particles, which may obstruct the carrier path and cause errors.
	**Warning!** Wear protective gloves when handling contaminated instruments. Gloved hands should be considered contaminated at all times; keep gloved hands away from eyes, mouth, nose, and ears.

	**Warning!** Mucous membranes are considered prime entry routes for infectious agents. Wear eye protection and a surgical mask when there is a possibility of aerosol contamination. Intact skin is generally considered an effective barrier against infectious organisms; however, small abrasions and cuts may not always be visible. Wear protective gloves when handling contaminated instruments.
	**Caution!** The buildup of deposits left by the evaporation of spilled fluids within the read chamber can impact measurements. Be sure to keep System Test records before and after maintenance so that changes can be noted.
	**Warning!** The instrument with all available modules weighs up to **85 lbs. (38.6 kg)**. Use two people when lifting and carrying the instrument.

Clean Exposed Surfaces

Exposed surfaces may be cleaned (not decontaminated) with a cloth moistened (not soaked) with water or water and a mild detergent. You'll need:

- Deionized or distilled water
- Clean, lint-free cotton cloths
- Mild detergent (optional)

1. Turn off and unplug the instrument.
2. Moisten a clean cotton cloth with water, or with water and mild detergent, then thoroughly wring it out so that liquid does not drip from it. **Do not soak the cloth.**
3. Wipe the plate carrier and all exposed surfaces of the instrument.
4. Wipe all exposed surfaces of the dispense module (if used).
5. If detergent was used, wipe all surfaces with a cloth moistened with water.
6. Use a clean, dry cloth to dry all wet surfaces.

Models with injectors: If the Tip Priming Trough overflows or other spills occur inside the instrument, wipe the carrier and the surface beneath the carrier with a dry cotton cloth. The internal chamber and probes are not customer-accessible. If overflow is significant, contact BioTek's Technical Assistance Center with any questions about your particular model.

Inspect/Clean Filter Cubes

Ambient laboratory air is used to cool the flash bulb, and the filter cubes can become dusty as a result. Filters should be inspected and cleaned at least every three months. You'll need:

- Isopropyl, ethyl, or methyl alcohol
- 100% pure cotton balls or high-quality lens-cleaning tissue
- Cloth gloves
- Magnifying glass

Do not touch the filters with your bare fingers!

1. Open the access door on the front of the instrument, and the side door, if equipped with a bottom filter cube. Slide the filter cubes out of their compartments.
2. Inspect the glass filters for speckled surfaces or a “halo” effect. This may indicate deterioration due to moisture exposure over a long period of time.

If you have any concerns about the quality of the filters, contact BioTek TAC.

3. Using cotton balls or lens-cleaning tissue moistened with a small amount of high-quality alcohol, clean each filter by lightly stroking its surface in one direction.
4. Use a magnifying glass to inspect the surface; remove any loose threads left from the cotton ball.
5. Replace the filter cubes and close the door.

Flush/Purge the Fluid Path

Applies only to Synergy Neo2 models with injectors.

At the end of each day that the dispense module is in use, flush the fluid path using the Gen5 priming utility. Leave the fluid to soak overnight or over a weekend, and then purge the fluid before using the instrument again.

This flushing and purging routine is also recommended before disconnecting the outlet tubes from the reader to prevent a spill. It is required before decontamination to remove any assay residue prior to applying isopropyl alcohol or sodium hypochlorite.

To flush the fluid path:

1. Fill two supply bottles with deionized or distilled water. Insert the supply (inlet) tubes into the bottles.
2. Place the priming plate on the carrier.
3. From the Gen5 main screen, select **System > Instrument Control > Synergy Neo2**.

4. Click the **Prime** tab and select **Dispenser 1**.
5. Set the Volume to **5000 µL**. Keep the default prime rate.
6. Click **Prime** to start the process. When the process is complete, carefully remove the priming plate from the carrier and empty it.
7. Repeat the process for Dispenser 2.

Leave the water in the system overnight or until the instrument will be used again. Purge the fluid from the system (see below) and then prime with the dispense reagent before running an assay.

To purge the fluid from the system:

1. Place the inlet tubes in empty supply bottles or a beaker.
2. Select **System > Instrument Control > Synergy Neo2**.
3. Click the **Prime** tab and select **Dispenser 1**.
4. Set the Volume to **2000 µL**.
5. Click **Purge** to start the process.
6. When the purge is complete, repeat the process for Dispenser 2.

After purging the system, you may wish to run a quick Dispense protocol to visually verify the dispense accuracy (see below) or the more thorough Dispense Accuracy and Precision Tests (see **Instrument Qualification**).

Run a Dispense Protocol (Optional)

Applies only to Synergy Neo2 models with injectors.

After flushing/purging the system and before running an assay that requires dispense, visually verify the dispense function.

1. Create a Dispense protocol in Gen5:
   a. Create a new protocol with the plate type set to match the plate you will use.
   b. Add a Dispense step with the following parameters:
      - Select Dispenser **1**.
      - Set Tip Priming to **Before this dispense step** and Volume to **10 µL**.
      - Set the Dispense Volume to **100 µL** (or an amount to match your assay protocol).

- Adjust the Rate to support the dispensing volume.
- Click **OK** to close the dialog and add the Dispense step to the procedure.

c. Add another Dispense step with the same parameters; select Dispenser **2**.

d. Add a Read step with the following parameters (Gen5 requires a Read step in a Dispense protocol):

- Select any Detection Method.
- Set the Read Type to **Endpoint.**
- Click **Full Plate**, click **Clear All**, then select well **A1**. Click **OK**.
- Select any wavelength or define one Filter Set.
- Click **OK** to close the dialog and add the Read step to the procedure.

e. Click **OK** to close the procedure.

f. Select **File > Save** and give the protocol an identifying name, such as “Dispense Observation.”

2. Fill the reagent bottles with a DI water–Tween solution (e.g., add 1 mL Tween 20 to 1000 mL of deionized water).
3. Create a new experiment using the "Dispense Observation" protocol.
4. Click **Read** and follow the prompts.
5. When the procedure is complete, visually assess the fluid level in the wells for accuracy. If the well volume appears to be unevenly distributed, clean the internal dispense tubes and injectors.

Empty/Clean the Tip Priming Trough

Applies only to Synergy Neo2 models with injectors.

The tip priming trough is a removable cup located in the rear pocket of the microplate carrier, used for performing the Tip Prime. The trough holds about 1.5 mL of liquid and must be periodically emptied and cleaned by the user. Gen5 will instruct you to do this at the start of an experiment that requires dispensing.

1. Extend the microplate carrier and carefully remove the tip priming trough from the carrier.
2. Wash the trough in hot, soapy water. Use a small brush to clean in the corners.
3. Rinse the trough thoroughly and allow it to dry completely.
4. Replace the trough in the microplate carrier.

Clean the Priming Plate

Applies only to Synergy Neo2 models with injectors.

Clean the priming plate regularly to prevent bacteria growth and residue buildup. Wash the plate in hot, soapy water, using a small brush to clean in the corners. Rinse thoroughly and allow it to dry completely.

Clean the Dispense Tubes and Injectors

Applies only to Synergy Neo2 models with injectors.

The Synergy Neo2's dispense tubes and injectors require routine cleaning, at least quarterly and possibly more frequently depending on the type of fluids dispensed.

Required Materials

- Protective gloves
- Safety glasses
- Mild detergent
- Clean, lint-free cotton cloths
- Deionized or distilled water
- Stylus (stored in a plastic cylinder affixed to the rear of the dispense module or reader) (PN 2872304)

Remove the Dispense Tubes and Injector Holders

1. Open the door on the front of the reader.
2. Grasp the injector tip holder by the tab and pull it up out of its socket.
3. Using your fingers, remove the thumbscrews securing the light shield to the top of the reader and slide the shield up the outlets tubes.
4. Slide the injector tip holder through the hole in the top of the reader.
5. Turn each tube's thumbscrew counterclockwise and gently pull each tube from its injector tip.
6. On the dispense module, turn each outlet tube's thumbscrew counterclockwise to disconnect it from the injector.

Clean the Dispense Tubes and Injectors

Some reagents can crystallize and clog the tubing and injectors. Daily flushing and purging can help to prevent this, but more rigorous cleaning may be necessary if reagent has dried in the tubing or injectors.

To clean the dispense tubes, soak them in hot, soapy water to soften and dissolve any hardened particles. Flush each tube by holding it vertically under a stream of water.

To clean the injectors:

1. Gently insert the stylus into each injector tip to clear any blockages. (The stylus is stored in a plastic cylinder affixed to the rear of the dispense module.)
2. Stream water through the pipe to be sure it is clean. If the water does not stream out, try soaking in hot, soapy water and then reinserting the stylus.

Be careful not to bend the injector tips. A bent tip might not dispense accurately.

Chapter 5

As-Needed Maintenance

This chapter contains maintenance and component-replacement procedures that need to be performed only occasionally.

Decontamination

Any laboratory instrument that has been used for research or clinical analysis is considered a biohazard and requires decontamination prior to handling.

Decontamination minimizes the risk to all who come into contact with the instrument during shipping, handling, and servicing. Decontamination is required by the U.S. Department of Transportation regulations.

Persons performing the decontamination process must be familiar with the basic setup and operation of the instrument.

	BioTek Instruments, Inc., recommends the use of the following decontamination solutions and methods based on our knowledge of the instrument and recommendations of the Centers for Disease Control and Prevention (CDC). Neither BioTek nor the CDC assumes any liability for the adequacy of these solutions and methods. Each laboratory must ensure that decontamination procedures are adequate for the biohazard(s) they handle.
	Wear prophylactic gloves when handling contaminated instruments. Gloved hands should be considered contaminated at all times; keep gloved hands away from eyes, mouth, and nose. Eating and drinking while decontaminating instruments is not advised.
	Mucous membranes are considered prime entry routes for infectious agents. Wear eye protection and a surgical mask when there is a possibility of aerosol contamination. Intact skin is generally considered an effective barrier against infectious organisms; however, small abrasions and cuts may not always be visible. Wear protective gloves when performing the decontamination procedure.

Required Materials

For all Synergy Neo2 models:

- Sodium hypochlorite (NaClO, or bleach)
- 70% isopropyl alcohol (as an alternative to bleach)
- Deionized or distilled water
- Safety glasses
- Surgical mask
- Protective gloves
- Lab coat
- Biohazard trash bags

- 125-mL beakers
- Clean, lint-free cotton cloths

Additional materials for models with the dispense module:

- Phillips screwdriver
- Small brush for cleaning the tip priming trough and priming plate
- (Optional) Mild detergent

Procedure for Models without a Dispenser

	The sodium hypochlorite (bleach) solution is caustic; wear gloves and eye protection when handling the solution. Do not immerse the instrument, spray it with liquid, or use a dripping-wet cloth. Do not allow the cleaning solution to run into the interior of the instrument. If this happens, contact the BioTek Service Department.
	Turn off and unplug the instrument for all decontamination and cleaning operations.

1. Turn off and unplug the instrument.
2. Prepare an aqueous solution of 0.50% sodium hypochlorite (bleach). If the effects of bleach are a concern, 70% isopropyl alcohol may be used.

Check the percent NaClO of the bleach you are using. Commercial bleach is typically 10.0% NaClO; prepare a 1:20 dilution. Household bleach is typically 5.0% NaClO; prepare a 1:10 dilution.

3. Wet a cloth with the bleach solution or alcohol, then thoroughly wring it out so that liquid does not drip from it. Do not soak the cloth.
4. Open the plate carrier door and slide out the plate carrier.
5. Wipe the plate carrier and all exposed surfaces of the instrument.
6. Wait 20 minutes. Moisten a cloth with deionized (DI) or distilled water and wipe all surfaces of the instrument that have been cleaned with the bleach solution or alcohol.
7. Use a clean, dry cloth to dry all wet surfaces.
8. Reassemble the instrument as necessary.

9. Discard the used gloves and cloths using a biohazard trash bag and an approved biohazard container.

Procedure for Models with a Dispenser

Perform the Routine Procedure when the Synergy Neo2 is functioning normally. If you are unable to perform a prime due to a system failure, perform the Alternate Procedure described on page 70.

Routine Procedure

If disinfecting with sodium hypochlorite (bleach), be sure to flush repeatedly with deionized water to remove the bleach. After disinfecting with sodium hypochlorite, perform the rinse procedure provided on page 69.

If disinfecting with alcohol, do not immediately prime with deionized water, because the drying effect of the alcohol is an important aspect of its disinfectant properties.

Clean Exposed Surfaces

1. Turn off and unplug the instrument.
2. Prepare an aqueous solution of 0.50% sodium hypochlorite (bleach). If the effects of bleach are a concern, 70% isopropyl alcohol may be used.

Check the percent NaClO of the bleach you are using. Commercial bleach is typically 10.0% NaClO; prepare a 1:20 dilution. Household bleach is typically 5.0% NaClO; prepare a 1:10 dilution.

3. Open the plate carrier door and slide out the plate carrier.
4. Wet a cloth with the bleach solution or alcohol, then thoroughly wring it out so that liquid does not drip from it. Do not soak the cloth.
5. Wipe the plate carrier and the exposed surfaces of the dispenser.
6. Wait 20 minutes. Moisten a cloth with deionized (DI) or distilled water and wipe all surfaces that have been cleaned with the bleach solution or alcohol.
7. Use a clean, dry cloth to dry all wet surfaces.
8. If the dispenser is installed, purge any fluid (see **Flush/Purge the Fluid Path** on page 59) and detach the outlet tubes from the instrument. If it is not installed,

attach only the dispenser's communication cable to the instrument. Remove the supply bottles and their holders.

9. Perform the decontamination procedures described below through page 70.

Decontaminate the Fluid Lines

1. Place a beaker with 20 mL of 0.5% sodium hypochlorite solution or 70% isopropyl alcohol near SYRINGE 1 on the dispenser.
2. Place the SYRINGE 1 inlet tube in the beaker.
3. If you have not already done so, detach the dispenser's outlet tubes from the instrument. Place the ends of the outlet tubes in an empty beaker and set the beaker next to the dispenser.
4. Launch Gen5 and from the main screen select **System > Instrument Control**, and click the **Prime** tab.
5. Select Dispenser **1**, enter a Volume of **5000 µL**, and keep the default dispense Rate.
6. Place the priming plate on the carrier.
7. Run two prime cycles, for a total of 10,000 µL.
8. Wait at least 20 minutes to allow the solution to disinfect the tubing.
9. Remove the inlet tube from the beaker of disinfectant solution.
10. From the Instrument Control dialog, change the Volume to 1000 µL.
11. Run one prime cycle, to flush the disinfectant out of the fluid lines.
12. Empty the beaker containing the outlet tubes. Put the tubes back in the empty beaker.
13. If sodium hypochlorite (bleach) was used, perform Rinse the Fluid Lines.

Otherwise (or after performing the Rinse procedure), repeat steps 1–13 for SYRINGE 2/Dispenser 2.

Rinse the Fluid Lines

Perform this procedure only if decontamination was performed using sodium hypochlorite.

1. Place a beaker containing at least 30 mL of deionized water on the dispenser.
2. Place the SYRINGE 1 or 2 inlet tube in the beaker.
3. If you have not already done so, place the outlet tubes in an empty beaker.
4. From the Instrument Control dialog, select Dispenser **1** or **2**, set the Volume to **5000 µL**, and keep the default dispense Rate.

5. Run five prime cycles, for a total of 25,000 µL.
6. Pause for 10 minutes and then run one prime cycle with 5000 µL. This delay will allow any residual sodium hypochlorite to diffuse into the solution and be flushed out with the next prime.
7. Empty the beaker containing the outlet tubes.
8. Wipe all surfaces with deionized water.
9. Discard the used gloves and cloths using a biohazard trash bag and an approved biohazard container.

Clean the Tubing and Injectors

Perform the procedures under **Clean the Dispense Tubes and Injectors** in **Preventive Maintenance**.

Decontaminate the Tip Priming Trough and Priming Plate

1. Remove the tip priming trough from the instrument's microplate carrier.
2. Wash the tip priming trough and priming plate in hot, soapy water. Use a small brush or cloth to clean the corners of the trough and plate.
3. To decontaminate, soak the trough and plate in a container of 0.5% sodium hypochlorite or 70% isopropyl alcohol for at least 20 minutes.
   - If decontaminating in a bleach solution, thoroughly rinse the trough and plate with DI water.
   - If decontaminating with alcohol, let the trough and plate air dry.
4. Discard the used gloves and cloths using a biohazard trash bag and an approved biohazard container.

Alternate Procedure

If you are unable to prime the Synergy Neo2 due to a system failure, decontaminate the instrument and the dispenser as follows:

1. Perform the procedures under **Clean the Dispense Tubes and Injectors** in **Preventive Maintenance**.
2. Prepare an aqueous solution of 0.50% sodium hypochlorite (bleach). If the effects of bleach are a concern, 70% isopropyl alcohol may be used.

Check the percent NaClO of the bleach you are using. Commercial bleach is typically 10.0% NaClO; prepare a 1:20 dilution. Household bleach is typically 5.0% NaClO; prepare a 1:10 dilution.

3. Slide the microplate carrier out of the instrument.
4. Wet a cloth with the bleach solution or alcohol, then thoroughly wring it out so that liquid does not drip from it. Do not soak the cloth.
5. Use the cloth to wipe:
   - All exterior surfaces of the instrument
   - All surfaces of the plate carrier
   - The exposed surfaces of the dispenser, including the syringe valves
6. Remove the tubing and the syringes from the dispenser and soak them in the bleach or alcohol solution. Wait for 20 minutes.
7. Moisten a cloth with DI or distilled water and wipe all surfaces that have been cleaned with the bleach solution or alcohol.
8. Rinse all tubing and the syringes with DI water.
9. Use a clean, dry cloth to dry all surfaces on the instrument and the dispenser.
10. Reassemble the dispenser as necessary.
11. Discard the used gloves and cloths using a biohazard trash bag and an approved biohazard container.

Dispenser Syringe Replacement

Refer to the **Preventive Maintenance** chapter for cleaning procedures you must perform regularly and also in the case of poor performance (for example, when Dispense Accuracy and Precision tests fail). If cleaning the dispenser does not eliminate performance problems, or if a syringe is obviously leaking, perform these instructions to replace a faulty syringe. Contact BioTek TAC to order replacement syringes.

To change a syringe, first use Gen5 to put the syringe in its maintenance position.

Syringe Maintenance Position

Do not change the syringe position or calibrate the dispensers unless instructed to do so as part of installation, upgrade, or maintenance.

Gen5 provides access to syringe setup functions for maintenance and calibration purposes. When a syringe needs to be installed or replaced, it must first be moved to its "maintenance position."

1. From the Gen5 main screen, select **System > Instrument Control > Synergy Neo2** and click the **Prime** tab.
2. Select the appropriate Dispenser number (**1** or **2**) associated with the syringe.
3. Click **Maintenance**. The syringe plunger will move to its furthest-from-home position. The syringe can then be disconnected from the drive bracket and unscrewed from the valve.

Replace the Syringe

After using Gen5 to move the syringe into its maintenance position:

1. Using your fingers, unscrew the bottom thumbscrew that secures the syringe, underneath the bracket. Retain this bottom thumbscrew; it is needed for the replacement syringe.
2. Unscrew the top thumbscrew to disengage the syringe from the valve.
3. Remove the new syringe from its protective box. (The syringe should already be assembled in one piece; if it is not, see "Install the Dispenser" in the **Installation** chapter.
4. Hold the syringe vertically with the threaded end at the top. Screw the top of the syringe into the bottom of the syringe valve. Finger-tighten only.
5. Carefully pull down the bottom of the syringe until it rests inside the hole in the bracket.
6. Pass the thumbscrew (used to hold the old syringe) up through this hole and thread it into the bottom of the syringe. Hold the syringe from rotating while tightening the thumbscrew. Finger-tighten only.
7. From the Gen5 main screen, select **System > Instrument Control > Synergy Neo2**. Click the **Prime** tab and click **Initialize**.

Chapter 6

Instrument Qualification Process

This chapter describes the tests that BioTek Instruments, Inc., has developed for complete qualification of all models of the Synergy Neo2. This chapter introduces the various test methods, describes the materials and relevant Gen5 protocols used to execute the tests, explains how to analyze test results, and provides troubleshooting tips in the event of a failure.

Instrument Qualification Procedures, starting on page 109, contains the actual step-by-step test procedures.

System Test

Each time the Synergy Neo2 is turned on, it automatically performs a series of tests on the reader's motors, lamp, the PMTs, and various subsystems. The duration of this system test depends on the reader model, and can take a few minutes to complete. If all tests pass, the microplate carrier is ejected and the green LED on the carrier switch remains on.

If any test results do not meet the internally coded Failure Mode Effects Analysis (FMEA) criteria established by BioTek, the reader beeps repeatedly and the red LED on the carrier switch flashes. If this occurs, press the carrier eject button to stop the beeping. If necessary, initiate another system test using Gen5 to try to retrieve an error code from the reader.

Refer to **Appendix B, Error Codes** starting on page 157, for information on error codes and for troubleshooting tips.

Refer to **Sample Reports**, on page 173, to see a sample System Test Report for Synergy Neo2.

Plate Shaker Test

This test verifies that the multispeed plate shaker is operating properly. The test involves creating and running a protocol with shaking enabled for a duration of 30 seconds. The sound of the carrier shaking is all that needs to be confirmed to verify that the plate shaker is operating properly.

Absorbance Testing Overview

For models with absorbance capability, BioTek developed a series of tests for the absorbance system using a combination of solid-state Absorbance Test Plates and liquid plates. The test plates and the materials used for creating the liquid plates are available for purchase from BioTek.

To qualify the absorbance system for the Synergy Neo2, you should perform:

- Absorbance Liquid Test 1 *and* Absorbance Plate Test (using BTI #7260522) *or*
- Absorbance Liquid Test 2

Optionally, to qualify operation in the UV range, you should also perform:

- Absorbance Liquid Test 3 *or* Absorbance Plate Test at 340 nm (using BTI #7260551)

BioTek Absorbance Test Plates

Absorbance Test Plate PN 7260522 uses NIST-traceable neutral density filters to confirm absorbance specifications in the visible range (400–800 nm). This test plate also contains precision-machined holes to verify mechanical alignment, and a glass filter in position C6 to test the wavelength accuracy of the monochromator-based absorbance system.

Absorbance Test Plate PN 7260551 uses NIST-traceable neutral density filters to confirm absorbance specifications in the UV range (340 nm).

Every test plate comes with a Test Plate Calibration Certificate, containing a table with Absorbance OD Standards for each filter at each wavelength supported by the plate. The certificate for test plate PN 7260522 also contains Wavelength Accuracy Standards tables with Expected Peak (nm) values with Test Ranges for the C6 glass filter.

Before the Absorbance Plate Test can be performed, the OD Standard values and Expected Peak/Test Range combinations must be entered into Gen5. Enter and save these values once initially, and then update them annually when the test plate is recertified by BioTek.

Test Methods

The Absorbance Plate Test is conducted using Gen5 software (**System > Diagnostics > Test Plates**) to confirm wavelength accuracy ("Peak Absorbance"); mechanical alignment; and optical density accuracy, linearity, and repeatability. When complete, Gen5 generates a results report displaying Pass or Fail for each individual test.

- **Peak Absorbance:** The BTI #7260522 test plate contains a glass filter in position C6 that is used to check the wavelength accuracy of the absorbance monochromator. The filter is scanned across a specified wavelength range in 1-nm increments. The wavelength(s) of maximum absorbance are compared to the expected peak wavelength(s) supplied on the test plate's data sheet. The accuracy of the wavelength should be ± 3 nm (±2 nm instrument, ±1 nm filter allowance).

- **Alignment:** The test plate has precisely machined holes to confirm mechanical alignment. The amount of light that shines through these holes is an indication of whether the microplate carrier is properly aligned with the absorbance optical path. A reading of more than 0.015 OD for any of the designated alignment holes indicates that the light is being "clipped" and the reader may be out of alignment.

- **Accuracy:** The test plate contains NIST-traceable neutral-density glass filters of known OD values at one or more wavelengths. Actual measurements are compared against the expected values provided in the test plate's data sheet. Since there are several filters with differing OD values, the accuracy across a range of ODs can be established. Once it is proven that the reader is accurate at these OD values, the reader is also considered to be linear. To further verify this, you can perform a linear regression analysis on the test plate OD values in a program such as Microsoft Excel; an R^2 value of at least 0.9900 is expected.

- **Repeatability:** This test ensures the instrument meets its repeatability specification by conducting repeated reads of each neutral-density filter on the test plate and comparing the results.

Sample Test Report

Refer to the **Sample Reports** appendix to see a sample Absorbance Plate Test Report for Synergy Neo2.

Troubleshooting

If a test fails, try the troubleshooting tips below. If the test continues to fail, contact BioTek TAC.

	**Important!** Do not remove filters from the Absorbance Test Plate. Do not use alcohol or other cleaning agents, and do not touch the filters with your bare fingers.
	If a higher-OD well reports "#N/A" for Min/Max Limit and Result, the measured OD is beyond the specified range for Accuracy or Repeatability used with this test, and therefore no pass/fail determination is made. It does not indicate a test failure.

Peak Absorbance Test

- Check the filter in the C6 position to ensure it is clean. If needed, clean the filter with lens paper. Do not remove the filter, and do not use alcohol or other cleaning agents.
- Verify that the Peak wavelength information entered for the plate in Gen5 matches the information provided on the test plate's data sheet.
- Check the calibration due date on the test plate's label. If the test plate is overdue for recalibration, contact BioTek to schedule service.
- Check the microplate carrier to ensure it is clear of debris.

Alignment Test

- Ensure that the test plate is properly seated in the microplate carrier.
- Check the four alignment holes (A1, A12, H1, H12) to ensure they are clear of debris.
- Check the microplate carrier to ensure it is clear of debris.

Accuracy Test

- Check the neutral-density filters to ensure they clean (positions C1, D4, E2, F5, G3, H6). If needed, clean the filters with lens paper. Do not remove any filters, and do not use alcohol or other cleaning agents.

- Verify that the wavelength/expected OD values entered for the plate in Gen5 match the information provided on the test plate's data sheet.
- Check the calibration due date on the test plate's label. If the test plate is overdue for recalibration, contact BioTek to schedule service.

Repeatability Test

- Check the neutral-density filters to ensure there is no debris that may have shifted between readings and caused changes.
- Check the microplate carrier to ensure it is clear of debris.

Absorbance Liquid Tests

BioTek Instruments, Inc., has developed a series of liquid test procedures for testing your reader's absorbance system.

Test Methods

Absorbance Liquid Test 1 confirms repeatability and alignment of the reader when a solution is used in the microplate. If these tests pass, then the lens placement and optical system cleanliness are proven. For the Repeatability portion of this test, two columns containing a color-absorbing solution are read five times at 405 nm. For each well, an "allowed deviation" is determined based on its Mean OD and the reader's repeatability specification. Each well's Standard Deviation must be less than its Allowed Deviation to pass. To confirm the reader’s mechanical alignment, the plate is rotated 180 degrees in the carrier (e.g., A1 is now in the H12 position) and the same two columns are read. The initial and new OD readings are compared, using the reader's accuracy specification. If the two readings in the same well do not meet specification, the reader may be out of alignment.

If an Absorbance Test Plate is not available, **Absorbance Liquid Test 2** may be conducted to test the instrument's alignment, repeatability, and accuracy by preparing a series of solutions of varying OD values as described on page 118.

Absorbance Liquid Test 3 is an optional test offered for those sites that must have proof of linearity at 340 nm. (Alternatively, the BioTek 340 nm Absorbance Test Plate may be used; see page 74) This test is optional since the Synergy Neo2 has good “front-end” linearity throughout the specified wavelength range. While the absolute values of the OD cannot be determined by this test, the results will indicate if there is adequate repeatable absorbance and a linear slope. This method is dependent upon proper dye dilution and a skilled pipetting technique. It is expected that the first dilution (mid-level solution) will have an absorbance value near 75% of that of the stock (high-level) solution, and that the second dilution (low-level solution) will have an absorbance value near 50% of that of the stock solution.

Gen5 Protocol Parameters

The information in this section represents the recommended reading parameters for the referenced Gen5 protocol(s). It is possible that your tests will require modifications to some of these parameters, such as the Plate Type.

> The Plate Type setting in each Gen5 protocol should match the actual plate in use.

Synergy Neo2 Abs Test 1.prt

Parameter	Setting
Plate Type	96 WELL PLATE
Two Read Steps	
Kinetic loop (one per Read step)	Set a Run Time/Interval combination to read the plate five times with minimal delay
Detection Method	Absorbance
Read Type	Endpoint
Optics Type	Monochromators
Read wells	First Read step: A1..H2 Second Read step: A11..H12
Wavelength	405 nm
Read Speed	Normal
Delay after plate movement	100 msec
Plate Out,In step between loops	Text "rotate the plate 180 degrees"

Synergy Neo2 Abs Test 2.prt

Parameter	Setting
Plate Type	96 WELL PLATE
Shake Step	Linear, 4 minutes, default frequency
Two Read Steps	
Kinetic loop (one per Read step)	Set a Run Time/Interval combination to read the plate five times with minimal delay
Detection Method	Absorbance
Read Type	Endpoint
Optics Type	Monochromators
Step labels	First Read step: "Normal" Second Read step: "Turnaround"
Read wells	Full plate
Wavelength	2 (450 nm, 630 nm)
Read Speed	Normal
Delay after plate movement	100 msec
Data Reduction	Define two Delta OD transformations (450 – 630 nm), one per Read data set

Synergy Neo2 Abs Test 3.prt

Parameter	Setting
Plate Type	96 WELL PLATE
Two Read Steps	
Kinetic loop (one per Read step)	Set a Run Time/Interval combination to read the plate five times with minimal delay
Detection Method	Absorbance
Read Type	Endpoint
Optics Type	Monochromators
Read wells	A1..H6
Wavelength	340 nm
Read Speed	Normal
Delay after plate movement	100 msec

Results Analysis

The Absorbance Liquid Test Procedures begin on page 116.

Absorbance specifications used with the liquid tests:

Repeatability Specification:

± 1.0% ± 0.005 OD from 0.000 to 2.000 OD

± 3.0% ± 0.005 OD from 2.000 OD to 2.500 OD

Accuracy Specification:

± 1.0% ± 0.010 OD from 0.000 to 2.000 OD

± 3.0% ± 0.010 OD from 2.000 OD to 2.500 OD

Absorbance Liquid Test 1

1. The plate is read five times in the “Normal” position at 405 nm. Calculate the Mean OD and Standard Deviation of those five reads for each well in columns 1 and 2.
2. For each well in columns 1 and 2, calculate the Allowed Deviation using the repeatability specification for a 96-well plate: ± 1% ± 0.005 OD from 0.000 to 2.000 OD (Mean * 0.010 + 0.005). For each well, its standard deviation should be less than its allowed deviation.

Example: Five readings in well A1 of 0.802, 0.802, 0.799, 0.798, and 0.801 result in a mean of 0.8004 and a standard deviation of 0.0018. The mean multiplied by 1.0% (0.8004 * 0.010) equals 0.008, and when added to 0.005 equals 0.013; this is the allowed deviation for well A1. Since the standard deviation for well A1 is less than 0.013, the well meets the test criteria.

3. The plate is read five times in the "Turnaround" position at 405 nm. Calculate the Mean OD of those reads for each well in columns 11 and 12.
4. Perform a mathematical comparison of the Mean values for each microwell in its Normal and Turnaround positions (that is, compare A1 to H12, A2 to H11, B1 to G12,… H2 to A11). To pass the test, the differences in the compared mean values must be within the accuracy specification for a 96-well microplate: ± 1.0% ± 0.010 OD from 0.000 to 2.000 OD.

   Example: If the mean value for well A1 in the Normal position is 1.902 with a specified accuracy of ± 1.0% ± 0.010 OD, then the expected range for the mean of the well in its Turnaround (H12) position is 1.873 to 1.931 OD. 1.902 x 0.010 + 0.010 = 0.029; 1.902 - 0.029 = 1.873; 1.902 + 0.029 = 1.931.

Absorbance Liquid Test 2

1. The plate is read five times at 450/630 nm ("Normal" position), resulting in five sets of Delta OD data. Calculate results for Linearity:
   a. Calculate the mean absorbance for each well, and average the means for each concentration.
   b. Perform a regression analysis on the data to determine if there is adequate linearity. Since it is somewhat difficult to achieve high pipetting accuracy when conducting linear dilutions, an R^2 value of at least 0.9900 is considered adequate.
2. Calculate the results for Repeatability:
   a. Calculate the Mean and Standard Deviation for the five readings taken at each concentration. Only one row of data needs to be analyzed.
   b. For each Mean below 2.000 OD, calculate the Allowed Deviation using the Repeatability specification for a 96-well plate of ±1.0% ±0.005 OD. (If above 2.000 OD, apply the ±3.0% ±0.005 specification.)
   c. The Standard Deviation for each set of readings should be less than the Allowed Deviation.
   d. Example: Readings of 1.950, 1.948, 1.955, 1.952, and 1.950 will result in a Mean of 1.951, and a Standard Deviation of 0.0026. The Mean (1.951) multiplied by 1.0% (1.951 x 0.010) = 0.0195, which, when added to the 0.005 (0.0195 + 0.005) = 0.0245 OD, which is the Allowed Deviation. Since the Standard Deviation is less than this value, the reader meets the test criteria.

3. After gathering data for the Linearity Test, the plate is read five more times with the A1 well in the H12 position ("Turnaround" position). This results in values for the four corner wells that can be used to assess alignment. Calculate results for the Alignment Test:

   a. Calculate the means of the wells A1 and H1 in the Normal plate position (data from Linearity Test) and in the Turnaround position.

   b. Compare the mean reading for well A1 to its mean reading when in the H12 position. Next, compare the mean values for the H1 well to the same well in the A12 position. The difference in the values for any two corresponding wells should be within the Accuracy specification for 96-well plates. If the four corner wells are within the accuracy range, the reader is in alignment.

      Example: If the mean of well A1 in the normal position is 1.902, where the specified accuracy is ±1.0% ±0.010 OD, then the expected range for the mean of the same well in the H12 position is 1.873 to 1.931 OD. (1.902 x 1.0% = 0.019 + 0.010 = 0.029, which is added to and subtracted from 1.902 for the range.)

Absorbance Liquid Test 3

1. The plate is read five times at 340 nm. For each well, calculate the Mean OD and Standard Deviation of the five readings.

2. For each Mean calculated in step 1, calculate the Allowed Deviation using the Repeatability specification for a 96-well plate (Mean OD x 0.010 + 0.005). For each well, its Standard Deviation should be less than its Allowed Deviation.

   Example: Five readings in well A1 of 0.802, 0.802, 0.799, 0.798, and 0.801 result in a Mean of 0.8004 and a Standard Deviation of 0.0018. The Mean multiplied by 1.0% (0.8004 * 0.010) equals 0.008, and when added to 0.005 equals 0.013; this is the Allowed Deviation for well A1. Since the Standard Deviation for well A1 is less than 0.013, the well meets the test criteria.

3. Calculate results for Linearity:
   - For each of the three test solutions, calculate the average Mean OD for the wells containing that solution (mean of wells A1 to H2, A3 to H4, and A5 to H6).
   - Perform a regression analysis on the data to determine if there is adequate linearity. The three average Mean OD values are the “Y” values. The solution concentrations are the “X” values (1.00, 0.75, 0.50). Since it is somewhat difficult to achieve high pipetting accuracy when conducting linear dilutions, an R^2 value of at least 0.9900 is considered adequate.

Troubleshooting

If an absorbance liquid test fails, try the following. If a test continues to fail, contact BioTek TAC.

- Check the microwells and plate carrier for debris that may have shifted and caused changes.
- Ensure the microplate is properly seated in the carrier.
- As applicable, confirm that the plate was properly oriented in the "Normal" and "Turnaround" positions.
- Liquid Test 1 can fail due to the meniscus effect, which can cause readings to decrease over time. If you suspect this may be the case, include a shake step between the read steps in the protocol.

Luminescence Test

For models with luminescence capability, BioTek uses the Harta Luminometer Reference Microplate to test the luminescence system. The test plate is LED-based and NIST-traceable. Contact BioTek to purchase a plate (BTI #8030015; includes microplate carrier adapters) or visit www.HartaInstruments.com to learn more.

Test Method

The Harta Luminometer Reference Microplate is used to determine a detection limit by leveraging a known correlation of 35 photons per attomole of ATP. By using the NIST data provided with the Harta plate in photons/s, a conversion factor of 0.02884 attomole/photon is applied to determine an ATP concentration and subsequent limit of detection for the instrument under test.

Gen5 Protocol Reading Parameters

The information in this section represents the recommended reading parameters for the referenced Gen5 protocol(s).

Neo 2_LumTest_Harta.prt

Parameter	Default Setting
Plate Type:	8030015 Harta - w/o 8032028 adapater
Delay Step:	3 minutes
Read Step 1:	
Detection Method:	Luminescence
Read Type:	Endpoint
Step Label:	Reference well A2
Read Wells:	A2
Light path 1:	LUM (Single PMT)
Optics position:	Top
Gain:	200
Integration Time:	0:10.00 MM:SS.ss
Delay After Plate Movement:	0 msec
Read Height:	10.00 mm
Read Step 2:	

Parameter	Default Setting
Detection Method:	Luminescence
Read Type:	Endpoint
Step Label:	Background
Read Wells:	D1..G4
Light path 1:	LUM (Single PMT)
Optics Position:	Top
Gain:	200
Integration Time:	0:10.00 MM:SS.ss
Delay After Plate Movement:	0 msec
Read Height:	10.00 mm
Read Step 3:	
Detection Method:	Luminescence
Read Type:	Endpoint
Read Wells:	A7–A8
Step Label:	Battery Check
Light path 1:	LUM (Single PMT)
Optics Position:	Top
Gain:	60
Integration Time:	0:01.00 MM:SS.ss
Delay After Plate Movement:	0 msec
Read Height:	10.00 mm

Results Analysis

The Luminescence Test procedure is described on page 121.

1. Determine if the plate's battery is functioning properly:
   - If A8 > (0.2 * A7), the battery is good. Otherwise, it requires replacement.

> A replacement battery is included with each Harta plate. A new spare battery will be supplied when the plate is recertified.

2. On the Harta plate's Calibration Certificate, locate the NIST measurement for the A2 position and convert it to attomoles: (A2 NIST measurement*0.02884)
3. Calculate the signal-to-noise ratio:
   (A2-Mean of the buffer cells)/(3 * Standard deviation of buffer cells)
4. Calculate the detection limit:
   A2 NIST measurement in attomoles/signal-to-noise ratio

Pass/Fail Criteria

- If the reader is equipped with the low-noise PMT, the detection limit must be **≤ 50 amol** to pass.
- If the reader is equipped with the red-shifted PMT, the detection limit must be **≤ 500 amol** to pass.

If you do not know which PMT is installed, (#49984 = low-noise PMT; #49721 = red-shifted PMT, please contact BioTek TAC.

Troubleshooting

If the luminescence test fails, try the following suggestions. If it continues to fail, print the results and contact BioTek TAC.

- Ensure that the reading is performed through a hole in the filter cube, not through a glass filter.
- Verify that the filter cube settings in Gen5 match the physical cube.
- If the test continues to fail, the optical probe(s) may need to be cleaned. Contact BioTek TAC for instructions.

Fluorescence Testing

For models with fluorescence capability, BioTek provides two options for testing the fluorescence system. One uses a solid state Fluorescence Test Plate (package BTI# 1400006*; contact BioTek Customer Care regarding availability). The other uses liquid plates, the materials for which are available for purchase from BioTek (see **Materials for Conducting Liquid Tests** on page 5).

*Fluorescence Test Plate BTI# 7092092 cannot be used for these tests.

BioTek Fluorescence Test Plate

The Fluorescence Test Plate simplifies the process for conducting fluorescence intensity, fluorescence polarization, and time-resolved fluorescence qualification tests on the Synergy

Neo2. The test plate is solid and therefore immune to the pipetting errors, evaporation issues, and costs experienced with conventional Liquid Tests.

The test plate package includes Gen5 protocols designed specifically for use with the test plate. The protocols include embedded Microsoft Excel spreadsheets to automatically calculate results and determine pass/fail. The protocols and their spreadsheets were fully validated in accordance with BioTek Instruments' Product Validation policies and procedures.

The package also contains a user guide that describes the test methods, helps you get started with using the plate, and provides important information for cleaning and maintaining the test plate. The guide also provides troubleshooting tips and information on the annual recalibration program.

Results Analysis

Refer to the Fluorescence Test Plate User Guide for descriptions of the data reduction calculations for each test. The tests must meet the following criteria to pass:

Fluorescence Intensity (FI) Tests	
Corners	%CV < 3.0
Linearity	R^2 >= 0.9500
Sensitivity, filter-based system:	
Top optics, Sodium Fluorescein analogue	Detection Limit <= 5.0 pM
Bottom optics, Sodium Fluorescein analogue	Detection Limit <= 5.0 pM
Top optics, Methylumbelliferone analogue	Detection Limit <= 160.0 pg/mL
Bottom optics, Methylumbelliferone analogue	Detection Limit <= 160.0 pg/mL
Sensitivity, monochromator-based system:	
Top optics, Sodium Fluorescein analogue	Detection Limit <= 20.0 pM
Bottom optics, Sodium Fluorescein analogue	Detection Limit <= 20.0 pM
Top optics, Methylumbelliferone analogue	Detection Limit <= 160.0 pg/mL
Bottom optics, Methylumbelliferone analogue	Detection Limit <= 160.0 pg/mL
Time-Resolved Fluorescence (TRF) Test	Detection Limit <= 250.0 fM
Fluorescence Polarization (FP) Test (filter-based system, dual PMT)	HPR Polarization > 340 mP LPR Standard Deviation < 5
Fluorescence Polarization (FP) Test (filter-based system, single PMT)	HPR Polarization > 340 mP LPR Standard Deviation < 5

Fluorescence Liquid Tests

Test Methods

- The **Corners Test** uses fluorescence compounds to verify that the plate carrier is properly aligned in relation to the fluorescence probe(s).
- The **Sensitivity Test** uses a fluorescence compound and buffer solution to test the fluorescence reading capability of the instrument. The ability to detect specific compounds at the required limit of detection ensures that the filters, optical path, and PMT(s) are all in working order. This test verifies that the difference between the concentration well under investigation and the mean of the median buffer well is statistically distinguishable.
- The **Linearity Test** verifies that the system is linear, that is, the signal changes proportionally with changes in concentration (R^2 value). Proving that the system is linear allows the Sensitivity Test to be run on two points instead of using serial dilutions.
- The **FP Test** verifies the ability of the instrument to measure polarization of the solution. It verifies the polarizers are installed in the proper orientation and the mechanism is in proper order.
- The **TRF Test** verifies the performance of the xenon flash bulb and that the filters, optical path, and PMTs are all in working order.

Gen5 Protocol Reading Parameters

The information in the following tables represents the recommended reading parameters. It is possible that your tests will require modifications to some of these parameters, such as the Plate Type (see **Troubleshooting Tips** on page 102).

> The Plate Type setting in each Gen5 protocol should match the plate you are actually using.

Synergy Neo 2_FI_T_SF.prt/Synergy Neo 2_FI_B_SF.prt

Parameter	Default Setting
Detection Method:	Fluorescence intensity
Read Type:	Endpoint
Plate Type:	Top Read: Costar 96 black opaque (#3915) Bottom Read: Greiner Sensoplate
Read Step 1:	

Parameter	Default Setting
Kinetic:	Run Time: 0:00:45 Interval: 0:00:03 Reads: 16
Step Label:	Sensitivity Read
Read Well:	D7
Filter Sets:	Single PMT Filter set 1: 485/528 Optics position: Top/Bottom Gain: (top and bottom optics) Auto, Scale to High Wells, D7, 50000
Read Speed:	Normal Delay after plate movement: 350 msec Measurements per data point: 50
Read Height:	5.75 mm (top read); 9.25 mm (bottom read)
Dynamic Range:	Standard
Light source:	Xenon Flash
Lamp energy:	Low (faster)
Read Step 2:	
Kinetic:	Run Time: 0:01:35 Interval: 0:00:06 Reads: 16
Step Label:	Sensitivity Read Buffer
Read Wells:	C9..E9
Filter Sets:	Single PMT Filter set 1: 485/528 Optics position: Top/Bottom Gain: (top and bottom optics) Auto, Use first filter set gain from FIRST Read Step
Read Speed:	Normal Delay after plate movement: 350 msec Measurements per data point: 50
Read Height:	5.75 mm (top read); 9.25 mm (bottom read)
Dynamic Range:	Standard
Light source:	Xenon Flash

Parameter	Default Setting
Lamp energy:	Low (faster)
Read Step 3:	
Step Label:	Corners Read
Read Wells:	A1–A3, A10–A12, H1–H3, H10–H12
Filter Sets:	Single PMT Filter set 1: 485/528 Optics position: Top/Bottom Gain: (top and bottom optics) Auto, Scale to High Wells, A3, 50000
Read Speed:	Normal Delay after plate movement: 350 msec Measurements per data point: 50
Read Height:	5.75 mm (top read); 9.25 mm (bottom read)
Dynamic Range:	Standard
Light source:	Xenon Flash
Lamp energy:	Low (faster)
Read Step 4:	
Step Label:	Linearity Read
Read Wells:	C1–F5
Filter Sets:	Single PMT Filter set 1: 485/528 Optics position: Top/Bottom Gain: (top and bottom optics) Auto, Scale to High Wells, C1, 50000
Read Speed:	Normal Delay after plate movement: 350 msec Measurements per data point: 50
Read Height:	5.75 mm (top read); 9.25 mm (bottom read)
Dynamic Range:	Standard
Light source:	Xenon Flash
Lamp energy:	Low (faster)

Synergy Neo 2_Single PMT_FP.prt and Synergy Neo 2_Dual PMT_FP.prt

This procedure contains one Read step using filters with Fluorescence Polarization enabled, inside a Plate Mode block.

Parameter	Default Setting
Detection Method:	Fluorescence polarization
Read Type:	Endpoint
Plate Type:	Costar 96 black opaque (#3915)
Synchronized Mode:	Plate Mode with Timing Control
Read Wells:	A6–H9
Filter Sets:	Single/Dual PMT FP 485/528 Optics position: Top Gain: Single PMT—Auto, Scale to well: A9, Scale value: 20000 Gain: Dual PMT—Side PMT1/Top PMT2: Auto, Scale to well: A9, Parallel scale value: 20000, Requested polarization: 20
Read Speed:	Normal
Delay After Plate Movement:	0 msec
Measurements Per Data Point:	50
Read Height:	6.50 mm
Dynamic Range:	Standard
Light source:	Xenon Flash
Lamp energy:	Low (faster)

Synergy Neo 2_TRF.prt

Parameter	Default Setting
Detection Method:	Time-resolved fluorescence
Read Type:	Endpoint
Plate Type:	Costar 96-well white opaque
Delay Step:	3 minutes

Parameter	Default Setting
Read Step 1:	
Kinetic:	Run Time: 0:00:15 Intervale: 0:00:01 Reads: 16
Step Label:	Sensitivity Read
Read Well:	A8
Filter Sets:	Single PMT 360/620 Optics position: Top Gain: Auto, Scale to High Wells, A8, 50000
Read Speed:	Normal
Delay after plate movement:	100 msec
Measurements per data point:	50
Read Height:	4.50 mm
Light source:	Xenon Flash
Lamp energy:	Low (faster)
Dynamic Range:	Standard
Delay:	300 µsec
Data collection time:	1000 µsec
Read Step 2:	
Kinetic:	Run Time: 0:00:45 Interval: 0:00:03 Reads: 16
Step Label:	Sensitivity Read Buffer
Read Wells:	A6–C6
Filter Sets:	Single PMT 360/620 Optics position: Top Gain: Auto, Use first filter set gain from FIRST Read Step
Read Speed:	Normal
Delay after plate movement:	100 msec
Measurements per data point:	50

Parameter	Default Setting
Read Height:	4.50 mm
Light source:	Xenon Flash
Lamp energy:	Low (faster)
Dynamic Range:	Standard
Delay:	300 µsec
Data collection time:	1000 µsec

Synergy Neo 2_M_FI_T_SF.prt and Synergy Neo 2_M_FI_B_SF.prt

Parameter	Default Setting
Detection Method:	Fluorescence
Read Type:	Endpoint
Plate Type:	Top: Costar 96 black opaque Bottom: Greiner SensoPlate
Read Step 1:	
Kinetic:	Run Time: 0:00:45 Interval: 0:00:03 Reads: 16
Step Label:	Sensitivity Read
Read Well:	D7
Wavelength:	Excitation: 485/14, Emission: 528/14
Optics Position:	Top/Bottom
Gain	Top: Auto, Scale to High Wells, D7, 50000
Read Speed:	Normal
Delay after plate movement:	100 msec
Measurements per data point:	50
Lamp Energy:	Low (faster)
Dynamic Range:	Standard
Read Height (for top optics):	5.00 mm
Read Step 2:	

Parameter	Default Setting
Kinetic:	Run Time: 0:01:35 Interval: 0:00:06 Reads: 16
Step Label:	Sensitivity Read Buffer
Read Wells:	C9–E9
Wavelengths:	Excitation: 485/14, Emission: 528/14
Optics Position:	Top/Bottom
Gain:	Top: Auto, Use first filter set gain from FIRST Read Step
Read Speed:	Normal
Delay after plate movement:	100 msec
Measurements per data point:	50
Lamp Energy:	Low (faster)
Dynamic Range:	Standard
Read Height (for top optics):	5.00 mm
Read Step 3:	
Step Label:	Corners Read
Read Wells:	A1–A3, A10–A12, H1–H3, H10–H12
Wavelengths:	Excitation: 485/14, Emission: 528/14
Optics Position:	Top/Bottom
Gain:	Top: Auto, Scale to High Wells, A3, 50000
Read Speed:	Normal
Delay after plate movement:	100 msec
Measurements per data point:	50
Lamp Energy:	Low (faster)
Dynamic Range:	Standard
Read Height (for top optics):	5.00 mm
Read Step 4:	
Step Label:	Linearity Read
Read Wells:	C1–F5

Parameter	Default Setting
Wavelengths:	Excitation: 485/14, Emission: 528/14
Optics Position:	Top
Gain:	Top: Auto, Scale to High Wells, C1, 50000
Read Speed:	Normal
Delay after plate movement:	100 msec
Measurements per data point:	50
Lamp Energy:	Low (faster)
Dynamic Range:	Standard
Read Height (for top optics):	5.00 mm

Neo 2_FI_T_MUB.prt and Neo 2_FI_B_MUB.prt

Parameter	Default Setting
Plate Type:	Top Optics: Costar 96-well black opaque (#3915) Bottom Optics: Greiner Sensoplate
Detection Method:	Fluorescence
Read Type:	Endpoint
Read Step 1:	
Kinetic	Run Time: 0:00:45 Interval: 0:00:03 Reads: 16
Step Label:	Sensitivity Read
Read Well:	D7
Filter Sets:	Single PMT 360/460 Optics position: Top/Bottom Gain: Auto, Scale to High Wells, D7, 80000
Read Speed:	Normal Delay after plate movement: 350 msec Measurements per data point: 50
Read Height:	4.50 mm (top read); 8.75 mm (bottom read)
Dynamic Range:	Standard
Light Source:	Xenon Flash

Parameter	Default Setting
Lamp Energy:	Low (faster)
Read Step 2:	
Kinetic:	Run Time: 0:01:35 Interval: 0:00:06 Reads: 16
Step Label:	Sensitivity Read Buffer
Read Well:	C9, D9, E9
Filter Sets:	Single PMT 360/460 Optic position: Top/Bottom Gain: Auto, Use first filter set gain from FIRST Read Step
Read Speed:	Normal Delay after plate movement: 350 msec Measurements per data point: 50
Read Height:	4.50 mm (top read); 8.75 mm (bottom read)
Dynamic Range:	Standard
Light Source:	Xenon Flash
Lamp Energy:	Low (faster)
Read Step 3:	
Step Label:	Linearity Read
Read Well:	C1–F5
Filter Sets:	Single PMT 360/460 Optics position: Top/Bottom Gain: Auto, Scale to High Wells, C1, 80000
Read Speed:	Normal Delay after plate movement: 350 msec Measurements per data point: 50
Read Height:	4.50 mm (top read); 8.75 mm (bottom read)
Dynamic Range:	Standard
Light Source:	Xenon Flash
Lamp Energy:	Low (faster)

Neo 2_M_FI_T_MUB.prt

Parameter	Default Setting
Plate Type:	Costar 96-well black opaque (#3915)
Detection Method:	Fluorescence
Read Type:	Endpoint
Read Step 1:	
Kinetic:	Run Time: 0:00:45 Interval: 0:00:03 Reads: 16
Step Label:	Sensitivity Read
Read Wells:	D7
Wavelengths:	Excitation: 360/14, Emission: 460/14
Optics Position:	Top
Gain:	Auto, Scale to High Wells, D7, 80000
Read Speed:	Normal
Delay after plate movement:	100 msec
Measurements per data point:	50
Lamp Energy:	Low (faster)
Dynamic Range:	Standard
Read Height:	4.50 mm
Read Step 2:	
Kinetic:	Run Time: 0:01:35 Interval: 0:00:03 Reads: 16
Step Label:	Sensitivity Read Buffer
Read Wells:	C9, D9, E9
Wavelengths:	Excitation: 360/14, Emission: 460/14
Optics Position:	Top
Gain:	Auto, Use first filter set gain from FIRST Read Step
Read Speed:	Normal
Delay after plate movement:	100 msec

Parameter	Default Setting
Measurements per data point:	50
Lamp Energy:	Low (faster)
Dynamic Range:	Standard
Read Height:	4.50
Read Step 3:	
Step Label:	Linearity Read
Read Wells:	C1–F5
Wavelengths:	Excitation: 360/14, Emission: 460/14
Optics Position:	Top
Gain:	Auto, Scale to High Wells, C1, 80000
Read Speed:	Normal
Delay after plate movement:	100 msec
Measurements per data point:	50
Lamp Energy:	Low (faster)
Dynamic Range:	Standard
Read Height:	4.50 nm

Results Analysis

The Fluorescence Liquid Test procedures begin on page 128.

Corners Test

1. Calculate the Mean of the 12 "corner" wells (A1–A3, A10–A12, H1–H3, and H10–H12).
2. Calculate the Standard Deviation for the same 12 wells.
3. Calculate the %CV: (Standard Deviation / Mean) * 100

The %CV must be **< 3.0** to pass.

Sensitivity Test

1. Calculate the Mean and Standard Deviation of the 16 reads for each of the buffer wells (C9, D9, E9).

2. Among the three buffer wells, find the Median Standard Deviation and corresponding Mean.
3. Calculate the Mean for the 16 reads of the SF Concentration well (D7).
4. Calculate the Signal-to-Noise Ratio (SNR) using the Mean SF Concentration, Buffer Media STD with its corresponding Buffer Mean:
(SF Mean - Buffer Mean)/3 * Buffer STD)
5. Calculate the Detection Limit:

Sodium Fluorescein: Using the known concentration value of SF and the calculated SNR: 1000/SNR

Filter-Based Fluorescence System		
Optics	**Filter Cube**	**To pass, the Detection Limit must be:**
Top	Cube 107 (or equivalent): 485/20, 528/20, 510 nm dichroic mirror	< 5 pM (<1 pM typical)
Bottom	Cube 107 (or equivalent): 485/20, 528/20, 510 nm dichroic mirror	< 5 pM (<2 pM typical)

Monochromator-Based Fluorescence System		
Optics	**Wavelength**	**To pass, the Detection Limit must be:**
Top/Bottom	EX 485 nm, EM 528 nm	<=20 pM (<=5 pM typical)

Methylumbelliferone: Using the known concentration value of MUB and the calculated SNR: 17/6.SNR

Filter-Based Fluorescence System:

Filter Cube	Optic Position	To pass, the Detection Limit must be:
Cube 107 (or equivalent)—360/40, 460/40, 400 nm dichroic mirror	Top/Bottom	<= 0.16 ng/mL (0.91 nM)

Monochromator-Based Fluorescence System:

Wavelengths	Optic Position	To pass, the Detection Limit must be:
Excitation—360 nm Emission—460 nm	Top	<= 0.16 ng/mL (0.91 nM)

Linearity Test

1. Calculate the Mean of the four wells for each concentration in columns 1–5.
2. Perform linear regression using these values as inputs:

Sodium Fluorescein: Filter- and Monochromator-Based Fluorescence System	
x	**y**
1000	Mean of the 1000 pM wells
500	Mean of the 500 pM wells
250	Mean of the 250 pM wells
125	Mean of the 125 pM wells
62.5	Mean of the 62.5 pM wells

Methylumbelliferone: Filter- and Monochromator-Based Fluorescence System	
x	**y**
100	Mean of the 100 nM wells
50	Mean of the 50 nM wells
25	Mean of the 25 nM wells
12.5	Mean of the 12.5 nM wells
6.25	Mean of the 6.25 nM wells

3. Calculate the R^2 value; it must be **>= 0.9500** to pass.

Fluorescence Polarization (FP) Test

1. Using the raw data from the Parallel read:
   - Calculate the Mean Blank (wells A6–H6).
   - Calculate the Signal for each HPR well: Subtract the Mean Blank from its measurement value.
   - Calculate the Signal for each LPR well: Subtract the Mean Blank from its measurement value.
2. Using the raw data from the Perpendicular read:
   - Calculate the Mean Blank (wells A6–H6)
   - Calculate the Signal for each HPR well: Subtract the Mean Blank from its measurement value.
   - Calculate the Signal for each LPR well: Subtract the Mean Blank from its measurement value.
3. Calculate the G-Factor for each LPR well:

   (Parallel LPR Sign * (1-0.02)) / (Perpendicular LPR Signal * (1+0.02))
4. Calculate the Mean G-Factor.
5. Calculate the Polarization value in mP for each HPR well ("PHPR"):

$$\frac{\text{Parallel HPR Signal - Mean G-Factor * Perpendicular HPR Signal * 1000}}{\text{Parallel HPR Signal + Mean G-Factor * Perpendicular HPR Signal}}$$

6. Calculate the Mean PHPR, in mP.

Filter Cubes	To pass, the Mean PHPR must be:
Dual PMT—Cube 61 (or equivalent); Single PMT—Cube 108 (or equivalent): 485/20, 528/20, 510 nm, dichroic mirror	> 340 mP

7. Calculate the Polarization value in mP for each LPR well ("PLPR"):

$$\frac{\text{Parallel LPR Signal - Mean G-Factor * Perpendicular LPR Signal * 1000}}{\text{Parallel LPR Signal + Mean G-Factor * Perpendicular LPR Signal}}$$

8. Calculate the Standard Deviation of the "PLPR," in mP.

Filter Cubes	To pass, the Standard Deviation of the PLPR::
Dual PMT—Cube 61 (or equivalent); Single PMT—Cube 108 (or equivalent): 485/20, 528/20, 510 nm dichroic mirror	< 5

Time-Resolved Fluorescence (TRF) Test

1. Calculate the Mean and Standard Deviation of the 16 reads for each of the buffer wells (A6, B6, C6).
2. Among the three buffer wells, find the Media Standard Deviation and corresponding Mean.
3. Calculate the Mean for the 16 reads of the Eu Concentration well (A8).
4. Calculate the Signal-to-Noise Ratio (SNR) using the Mean Eu Concentration and Buffer Median STD with its corresponding Buffer Mean:
   (Eu Mean – Buffer Mean)/(3 * Buffer STD)
5. Calculate the Detection Limit, in fM:
   20000/(Mean Eu - Mean DI water)/(3 * Standard Deviation DI water)

Filter Cube	To pass, the Detection Limit must be:
Cube 112 (or equivalent): 360/40, 620/40, 400 nm dichroic mirror	<= 250 fM

Troubleshooting

If any tests fail, please try the following suggestions. If the test(s) continue to fail, print the results and contact BioTek's Technical Assistance Center.

- Are the solutions fresh? Discard the plate and any opened, unused test solutions after seven days.
- Are the excitation/emission filters clean?
- Are you using the proper filter cube?
- If the Corners Test continues to fail, the hardware may be misaligned. Contact BioTek TAC.
- Are you using new/clean plates? If the base of a clear-bottom plate is touched, clean the entire base with alcohol (95% ethanol) and then wipe with a lint-free cloth. Before placing the plate in the instrument, blow the bottom of the plate with an aerosol duster. If the test fails again, the optical probe(s) may need to be cleaned. Contact BioTek TAC.
- Review the pipetting instructions to verify the plate was correctly prepared.
- Does the Plate Type setting in the Gen5 protocol match the plate you used?
- For models with a dispenser, spilled fluid inside the reader may be fluorescing, which can corrupt your test results. If you suspect this is a problem, contact BioTek TAC.
- When testing Fluorescence Polarization capability using a solid black plastic microplate, if the standard deviation for the buffer wells is too high, try moving the buffer wells to another column. With some black plastic plates, the wells in the center of the plate may be slightly distorted due to the plate molding process, and this can affect the standard deviation.
- The Read steps in the protocols use the Gen5 Automatic Gain Adjustment feature to determine optimum sensitivity values for the plate. If an Auto Gain Result value is outside the range of 30–200, this may indicate a problem.

  If the value is less than 30:

  - The stock solution/dilution concentrations may be too high. Try creating fresh solutions/dilutions, and rerun the test using a new, clean plate.
  - If all of the tests are passing but the Gain value is low, a PMT in your reader may just be very sensitive. Contact BioTek's Technical Assistance Center to confirm that this may be the case.

  If the value is greater than 200:

  - The stock solution/dilution concentrations may be too low. Try creating fresh solutions/dilutions, and rerun the test using a new, clean plate.
  - For injector models, spilled fluid inside the reader may be fluorescing, which can corrupt your test results. If you suspect this is a problem, contact BioTek TAC.
  - The PMTs or optical path(s) may be deteriorating, or the optics or other hardware may be misaligned. Contact BioTek's Technical Assistance Center.

Injection System Testing

For models equipped with injectors and an external dispense module, BioTek has developed a set of tests to ensure that the injection system performs to specification.

Test Method

- The **Accuracy Test** is a measure of the mean volume per well for multiple dispenses. The actual weight of the dispensed fluid is compared to the expected weight and must be within a certain percentage to pass. Pass/Fail criteria depends on the per-well volume dispensed: 2.0% for 80 µL, 5.0% for 20 µL, and 20.0% for 5 µL. It is assumed that one gram is equal to one milliliter.

  The test uses a single green dye test solution and a 96-well microplate (per injector) to test the three different volumes. The balance is tared with the empty plate, and then the 80 µL dispense is performed for columns 1–4. The fluid is weighed and the balance is tared again (with the plate on the balance). This process is repeated for the 20 µL and 5 µL dispenses. It is assumed that the solutions used are at room temperature. A precision balance (three-place) is used to weigh the plate.
- The **Precision Test** is a measure of the variation among volumes dispensed to multiple wells. For each volume dispensed (80 µL, 20 µL, and 5 µL) to four columns, the %CV (coefficient of variation) of 32 absorbance readings is calculated. Pass/Fail criteria depends on the per-well volume dispensed: 2.0% for 80 µL, 7.0% for 20 µL, and 10.0% for 5 µL. The plate is read in an absorbance reader at 405/750 nm for columns 1–4 and at 630/750 nm for columns 5–12.

The two tests are performed simultaneously and use the same plate.

Gen5 Parameters

The information in this section represents the recommended reading parameters for the referenced Gen5 protocol(s). It is possible that your tests will require modifications to some of these parameters, such as the Plate Type.

> The Plate Type setting in each Gen5 protocol should match the actual plate in use.

Synergy Neo2 Disp 1 Test.prt and Synergy Neo2 Disp 2 Test.prt

Parameter	Setting
Plate Type	96 WELL PLATE

Parameter	Setting
Dispense Step	Dispenser <select 1 or 2, depending on the protocol> Dispense to wells A1..H4 Tip prime before this dispense step, 20 µL Dispense 80 µL at rate 275 µL/sec
Plate Out,In	Suggested comment: Weigh the plate (80 µL test). RECORD the weight, TARE the balance. Place the plate back on the carrier. Click **OK** to continue.
Dispense	Dispenser <select 1 or 2, depending on the protocol> Dispense to wells A5..H8 | Tip prime before this dispense step, 20 µL Dispense 20 µL at rate 250 µL/sec
Plate Out,In	Suggested comment: Weigh the plate (20 µL test). RECORD the weight and TARE the balance. Place the plate back on the carrier. Click **OK** to continue.
Dispense	Dispenser <select 1 or 2, depending on the protocol> Dispense to wells A9..H12 Tip prime before this dispense step, 5 µL Dispense 5 µL at rate 225 µL/sec
Plate Out,In	Suggested comment: Weigh the plate (5 µL test). RECORD the weight. PIPETTE 150 µL/well of DI water into all 12 columns. Place the plate back on the carrier. Click **OK** to perform the Read steps.
Shake	Orbital at 425 cpm (3 mm) for 30 seconds.
Read	Step label: “80 ul Read_Disp 1” (or _Disp 2) Wells: A1..H4 Detection Method: Absorbance Read Type: Endpoint Read Speed: Normal Two Wavelengths: 405 and 750 nm
Read	Step label: “20 and 5 ul Read_Disp 1” (or _Disp 2) Wells: A5..H12 Detection Method: Absorbance Read Type: Endpoint Read Speed: Normal Two Wavelengths: 630 and 750 nm

Results Analysis

The Injection System Test procedure begin on page 138.

When the experiment for one injector is complete, 32 delta OD values are reported for each of the three dispense volumes. The pass/fail criteria for each set of 32 wells with the same dispense volume is based on the calculated coefficient of variation (% CV) and Accuracy % Error.

For each volume dispensed (80 µL, 20 µL, 5 µL), for each injector (1, 2):

1. Calculate the Standard Deviation of the 32 wells.
2. Calculate the Mean of the 32 wells.
3. Calculate the %CV: (Standard Deviation/Mean) * 100
4. Calculate the Accuracy % Error:
   ((Actual Weight – Expected Weight)/Expected Weight) * 100

Expected Weights for 32 wells: 80 µL (2.560 g), 20 µL (0.640 g), 5 µL (0.160 g). It is assumed that one gram is equal to one milliliter.

Dispense Volume	To pass, %CV must be	To pass, Accuracy % Error must be:
80 µL	≤ 2.0%	≤ 2.0%
20 µL	≤ 7.0%	≤ 5.0%
5 µL	≤ 10.0%	≤ 20.0%

If any tests fail, prime the fluid lines and rerun the tests. If the tests fail again, the injectors may require cleaning; see Clean the Dispense Tubes and Injectors on page 62. If tests continue to fail, contact BioTek TAC.

Alpha Detection Test

The alpha laser has been factory-calibrated to meet specification. BioTek Instruments, Inc., has developed a set of test protocols that can be used with Alphascreen Omnibeads to verify the functionality of the alpha laser system. Because the detector for the alpha system is functionally and optically identical to the luminescence system, the luminescence test may be used to verify detector functionality.

The **Crosstalk** test is a measure of how well the optical system can distinguish the signals emitted from the well being read from those of any adjacent well. This test also determines the signal-to-noise ratio (SNR) of the test plate and verifies that the signal is at an acceptable level for the sample material used. The test is designed for use with Alphascreen Omnibeads, and it is assumed that 96-well plates are used with 100 µL well volumes.

Gen5 Protocol Reading Parameters

The information in the following table represents the recommended reading parameters. It is possible that your tests will require modifications to some of these parameters, such as Plate Type or Gain value (see **Troubleshooting Tips** above).

> The Plate Type setting in the Gen5 protocol should match the plate you are actually using.

Synergy Neo2_AlphaTest_Crosstalk.prt

This procedure contains one read step and calculates crosstalk based on the full plate data.

Parameter	Default Setting
Plate Type	96-well
Detection Method	Alpha
Read Type	Endpoint
Read Wells	Full plate
Gain	120
Delay after plate movement	0 msec
Excitation time	100 msec
Delay after excitation	120 msec
Integration time	100 msec
Read height	7.00 mm

Results Analysis

1. Calculate the crosstalk for each of the four wells of Omnibead solution by dividing the background-subtracted Mean value of the surrounding adjacent wells by the background-subtracted Omnibead suspension well.
2. Average the % crosstalk of the four test wells to determine level of crosstalk (Crosstalk Mean).
3. Verify that the % crosstalk is less than 0.1%
4. Calculate the signal-to-noise ratio (SNR) by using the following equation:
   SNR = (signal mean – background mean)/(SQRT(signal STD² + background STD²))
5. Verify that SNR is greater than **10**.

Troubleshooting Alpha Tests

If the test fails, please try the following suggestions. If the test(s) continue to fail, print the results and contact BioTek's Technical Assistance Center.

- Are the solutions fresh?
- Have the solutions been stored properly (between +2°C and +6°C)?
- Has the kit been exposed to excessive light (in excess of 100 lux)?
- If the Crosstalk test continues to fail, the laser may not be firing. Contact BioTek TAC.

Chapter 7

Instrument Qualification Procedures

This chapter contains the step-by-step procedures for verifying that the Synergy Neo2 and its various subsystems are performing to specification.

Instrument Qualification Process, starting on page 73, introduces the various test methods, describes the materials and relevant Gen5 protocols used to execute the tests, explains how to analyze test results, and provides troubleshooting tips in the event of a failure.

Instrument Qualification Overview

This chapter contains BioTek Instrument's recommended qualification procedures for all Synergy Neo2 models.

Every Synergy Neo2 reader and external dispense module is fully tested at BioTek prior to shipment and should operate properly upon initial setup. If you suspect that a problem occurred during shipment, if you have received the equipment after returning it to the factory for service, and/or if regulatory requirements dictate that you qualify the equipment on a routine basis, perform the procedures outlined in this chapter.

See the **Recommended Qualification Schedule** on page 111 to determine which qualification tests shall be conducted for your Synergy Neo2 model and to meet your site's regulatory requirements.

A Product Qualification Package (BTI # 1350526) for the Synergy Neo2 is available for purchase. The package contains complete procedures, Gen5 protocols, checklists, and logbooks for performing Installation Qualification, Operational Qualification, Performance Qualification, and Preventive Maintenance. Contact your local BioTek dealer for more information.

If the gas controller module is used with the Synergy Neo2, refer to the *Gas Controller User Guide* (BTI #1211000) or Gas Controller Product Qualification Package (BTI #1210512) for qualification procedures.

IQ/OQ/PQ Description

Installation Qualification confirms that the reader and its components have been supplied as ordered and ensures that they are assembled and configured properly for your lab environment.

- The recommended IQ procedure consists of setting up the instrument and its components as described in the Installation chapter, and performing the System Test. For models with injectors, a quick test with fluid is also performed, to ensure that the dispense module is properly installed and there are no leaks.
- The IQ procedure should be performed before the reader is used for the first time). The successful completion of the IQ procedure verifies that the instrument is installed correctly.

Operational Qualification confirms that the equipment operates according to specification initially and over time.

- The recommended OQ procedure consists of performing the system test, Absorbance Plate Test, a series of Fluorescence Tests, and, if the external dispense module is used, Dispense Accuracy and Precision Tests.

- The OQ procedure should be performed initially (before first use) and then routinely; the recommended interval is annually. It should also be performed after any major repair or upgrade to the hardware or software.
- Although out-of-tolerance failures will be detected by the OQ tests, results should be compared with those from the routine Performance Qualification tests and previous OQ tests to monitor for trends.
- The successful completion of the OQ procedure, in combination with results that are comparable to previous PQ and OQ tests, confirms that the equipment is operating according to specification initially and over time.

Performance Qualification confirms that the reader consistently meets the requirements of the tests performed at your laboratory.

- The recommended PQ procedure consists of performing the System Test, Absorbance Plate Test, a series of fluorescence tests, and, if the external dispense module is used, Dispense Accuracy and Precision Tests. Your facility's operating policies may also require that you routinely perform an actual assay, to confirm that the reader will consistently give adequate results for the assays to be run on it.
- These tests should be performed routinely; the recommended interval is monthly or quarterly, depending on the test. This frequency may be adjusted depending on the trends observed over time.
- The successful completion of the PQ procedure confirms that the equipment is performing consistently under normal operating conditions.

Recommended Qualification Schedule

This table defines BioTek-recommended intervals for qualifying a Synergy Neo2 used two to five days a week. The actual frequency, however, may be adjusted depending on your usage of the instrument and its various models. The schedule assumes that the instrument is properly maintained as outlined in the **Preventive Maintenance** chapter.

Tasks/Tests	IQ	OQ	PQ	
	Initially	Initially/ Annually	Monthly	Quarterly
All models:				
Installation, setup, and configuration of the reader, host computer, and Gen5 software	✓			
System Test	✓	✓	✓	
Models with absorbance capability:				
Absorbance Plate Test		✓	✓	
Absorbance Liquid Test 1 or Liquid Test 2*		✓		✓
(Optional) Absorbance Liquid Test 3 *or* 340 nm Absorbance Plate Test (using BTI #7260551)		✓		✓
Models with fluorescence capability:				
Corners, Sensitivity, Linearity (FI) Tests		✓	✓	
Fluorescence Polarization (FP) Test		✓		✓
Time-Resolved Fluorescence (TRF) Test		✓		✓
Models with luminescence capability:				

Tasks/Tests	IQ	OQ	PQ	
	Initially	Initially/ Annually	Monthly	Quarterly
Luminescence Test		✓	✓	
Models with injectors and an external dispense module:				
Installation and setup of external dispense module	✓			
Injection System Test	✓			
Dispense Accuracy/Precision Test		✓		✓
Alpha Laser capability:				
Alpha Detection Test		✓		

* If you have Absorbance Test Plate BTI #7260522, perform Liquid Test 1. Otherwise, perform Liquid Test 2.

System Test

Instrument System Test, starting on page 74, describes this test and explains where to find information on error codes and troubleshooting tips, as well as sample test reports for Synergy Neo2.

Setup

If your assays use incubation, we recommend enabling temperature control for at least 37°C and allowing the incubator to reach its set point before running the System Test. To access this feature, select **System > Instrument Control** and click the **Pre-Heating** tab.

Test Procedure

1. From the Gen5 main screen, select **System > Diagnostics > Run System Test**.

The duration of the test depends on the reader model; it can take a few minutes to complete.

If the test fails during execution, a message box appears in Gen5. Close the box; the System Test Report will contain the error code that was generated by the failure.

2. When the test is complete, a dialog appears, requesting additional information. Enter any required information and then click **OK**.
3. The test report appears; it shows either "SYSTEM TEST PASS" or "SYSTEM TEST FAIL *** ERROR (error code) DETECTED."

   If the test failed, go to page 157 to look up the error code and determine its cause. If the cause is something you can fix, turn off the reader, fix the problem, and then turn the reader back on and retry the test. If the test continues to fail, or if the cause is not something you can fix, contact BioTek TAC.
4. If required, print, sign, and date the report, and store it with your test documentation.
5. If applicable, turn off the incubator.

Absorbance Plate Tests

BioTek Absorbance Test Plates, starting on page 74, describes the test methods and provides troubleshooting tips in the event of a test failure.

Requirements

To perform this test, you will need:

- Absorbance Test Plate, BTI #7260522
- (Optional) 340 nm Absorbance Test Plate, BTI #7260551
- Current Absorbance Test Plate Calibration Certificate(s)

Setup

Before an Absorbance Test Plate can be used for qualification, you must enter information from its Calibration Certificate into Gen5. Perform these steps initially, and then repeat them annual after the test plate is recertified by BioTek:

1. Obtain the current Test Plate Calibration Certificate.
2. Start Gen5 and select **System > Diagnostics > Test Plates > Add/Modify Plates**.
3. Click **Add**. The Absorbance Test Plate dialog appears.
4. Select the appropriate Plate Type and then enter the plate's serial number.
5. Enter the Last Certification and Next Certification dates from the calibration label on the Test Plate.
6. If the wavelength values in the top row of the grid in Gen5 are appropriate for your tests, enter the OD Standard values from the Calibration Certificate into the grid. Make sure you enter the correct value for each well/wavelength combination.

   If you need to change the wavelength values, click **Wavelength List**. Add, change, or delete the values as needed and click **OK**.

7. If applicable: Select the number of Peak Wavelength tests to run (up to 4), based on the desired Expected Peak wavelengths provided on the Calibration Certificate. Enter the Expected Peak value(s) from the Certificate and set the Test Range – and + values.

   Depending on the manufacture date of the test plate, the glass type may be Erbium, Holmium, or Didymium. Contact BioTek TAC if you are not sure which glass type is used in your plate.

   - If the C6 filter is Erbium or Holmium glass, the certificate contains two Spectral Bandpass tables. For wavelengths greater than 285 nm, we recommend performing the test using the **5.0 nm** table. For wavelengths in the 230–285 nm range, we recommend using the 2.4 nm table.

     Erbium: Any peak value in the table can be used.

     Holmium: For best results, use the expected peak values closest to 242, 279, 362, 417, and 538 nm.
   - If the C6 filter is Didymium glass, the certificate provides a single peak wavelength value. Enter this value into Gen5 and set the Test Range – and + values so the range displayed in parentheses is "(580 to 590)".

8. Review all of the values that you entered. When finished, **click** OK to save the information.

Test Procedure

1. In Gen5, select **System > Diagnostics > Test Plates > Run**. If prompted, select the desired Test Plate and click **OK**.
2. When the Absorbance Test Plate Options dialog appears, enter any required information.
3. If applicable, check **Perform Peak Wavelength Test**.
4. Highlight the wavelength(s) to be included in this test. Select only those wavelengths most appropriate for your use of the reader.
5. (Optional) Enter a comment.
6. Click **Start Test**.
7. Place the Absorbance Test Plate on the microplate carrier, with A1 in the proper location.
8. Click **OK** to run the test.
9. When the test completes, the results report will appear. Scroll down through the report; every result should show "PASS".
   - Troubleshooting tips are provided on page 76.
   - Test descriptions are provided on page 75.

Absorbance Liquid Tests

Absorbance Liquid Tests, starting on page 77, describes the test methods, lists the Gen5 protocol parameters, explains how to analyze the test results, and provides troubleshooting tips in the event of test failure.

Absorbance Liquid Test 1

The tests in this section require specific microplates, solutions, and wavelengths. Your laboratory may require a deviation from some of these tests. For example, you may wish to use a different plate, test solution, and/or wavelength. If deviation from the tests as presented in this section is required, perform the following steps the first time each test is run:

- Perform the tests exactly as described in the following pages.
- Rerun the tests using your particular plates, solutions, wavelengths, and so on. If results are comparable, then the results from these tests will be your baseline for future tests.
- Document your new test procedure(s) and save all test results.

Materials

Manufacturer part numbers are subject to change.

- New 96-well, clear, flat-bottom microplate (Corning Costar #3590 recommended)
- Stock Solution A or B, which may be formulated by diluting a dye solution available from BioTek (A) or from the materials listed below (B)
- Gen5 protocol **Synergy Neo2 Abs Test 1.prt**, described on page 78

Solution A

- BioTek QC Check Solution No. 1 (PN 7120779, 25 mL; PN 7120782, 125 mL)
- Deionized water
- 5-mL Class A volumetric pipette
- 100-mL volumetric flask

1. Pipette a 5-mL aliquot of BioTek QC Check Solution No. 1 into a 100-mL volumetric flask.
2. Add 95 mL of DI water; cap and shake well. The solution should measure approximately 2.000 OD when using 200 µL in a flat-bottom microwell.

Solution B

- Deionized water
- FD&C Yellow No. 5 dye powder (typically 90% pure)

- Tween 20 (polyoxyethylene (20) sorbitan monolaurate) or BioTek wetting agent (PN 7773002) (a 10% Tween solution)
- Precision balance with capacity of 100 g minimum and readability of 0.001 g
- Weigh boat
- 1-liter volumetric flask

1. Weigh out 0.092 g of FD&C Yellow No. 5 dye powder into a weigh boat.
2. Rinse the contents into a 1-liter volumetric flask.
3. Add 0.5 mL of Tween 20, or 5 mL of BioTek's wetting agent.
4. Fill to 1 liter with DI water; cap and shake well. The solution should measure approximately 2.000 OD when using 200 µL in a flat-bottom microwell.

Test Procedure

Be sure to use a new microplate. Debris, fingerprints, or scratches may cause variations in readings.

1. Using freshly prepared stock solution (Solution A or B), prepare a 1:2 dilution using deionized water (one part stock, one part deionized water; the resulting solution is a 1:2 dilution).
2. Pipette 200 µL of the concentrated solution (A or B) into the first column of wells in the microplate.
3. Pipette 200 µL of the diluted solution into the second column of wells.
4. Create a Gen5 experiment based on the **Synergy Neo2 Abs Test 1** protocol and read the plate. When prompted, rotate the plate 180 degrees and continue.
5. When the experiment is finished:
   - Save the experiment. Refer to the instructions on page 80 to perform calculations and determine pass/fail.
   - Troubleshooting tips are provided on page 83.
   - Test descriptions are provided on page 77.

Absorbance Liquid Test 2

The recommended method for testing the instrument's alignment, repeatability, and accuracy is to use Absorbance Test Plate BTI #7260522 (see page 74). If the test plate is not available, however, Liquid Test 2 can be used for these tests.

Materials

Manufacturer part numbers are subject to change.

- A new 96-well, clear, flat-bottom microplate (Corning Costar #3590 is recommended)
- Ten test tubes, numbered consecutively, set up in a rack
- Calibrated hand pipette (Class A volumetric pipette recommended)
- Solution A or B (see the instructions for Liquid Test 1)
- A 0.05% solution of deionized water and Tween 20
- Gen5 protocol **Synergy Neo2 Abs Test 2.prt**, described on page 79

Test Procedure

1. Create a percentage dilution series, beginning with 100% of the original concentrated stock solution (A or B) in the first tube, 90% of the original solution in the second tube, 80% in the third tube, all the way to 10% in the tenth tube. Dilute using the 0.05% solution of deionized water and Tween 20. This solution can also be made by diluting the BioTek wetting agent 200:1.

Tube Number	1	2	3	4	5	6	7	8	9	10
Volume of original concentrated solution (mL)	20	18	16	14	12	10	8	6	4	2
Volume of 0.05% Tween solution (mL)	0	2	4	6	8	10	12	14	16	18
Absorbance expected if original solution is 2.0 at 200 µL	2.0	1.8	1.6	1.4	1.2	1.0	0.8	0.6	0.4	0.2

The choice of dilutions and the absorbance of the original solution can be varied. Use this table as a model for calculating the expected absorbances of a series of dilutions, given a different absorbance of the original solution.

2. Pipette 200 µL of the concentrated solution from Tube 1 into each well of the first column, A1 to H1, of a new flat-bottom microplate.
3. Pipette 200 µL from each of the remaining tubes into the wells of the corresponding column of the microplate (Tube 2 into wells A2 to H2, Tube 3 into wells A3 to H3, and so on).

4. Create a Gen5 experiment based on the **Synergy Neo2 Abs Test 2** protocol and read the plate. When prompted, rotate the plate 180 degress.
5. When finished:
   - Save the experiment. Refer to the instructions on page 81 to perform calculations and determine pass/fail.
   - Troubleshooting tips are provided on page 83.
   - Test descriptions are provided on page 77.

Absorbance Liquid Test 3

Absorbance Liquid Test 3 is provided for sites requiring proof of linearity at 340 nm. This test is optional because the Synergy Neo2 has good "front end" linearity throughout its wavelength range. As an alternative, the 340 nm Absorbance Test Plate (BTI #7260551) may be used for this test.

Materials

Manufacturer part numbers are subject to change.

- New 96-well, clear, flat-bottom microplate (Corning Costar #3590 recommended); alternatively, a UV transparent microplate may be used
- Calibrated hand pipette(s)
- Beakers and graduated cylinder
- Precision balance with readability to 0.01 g
- Buffer solution described below
- Gen5 protocol **Synergy Neo2 Abs Test 3.prt**, described on page 80

Buffer Solution

- Deionized water
- Phosphate-Buffered Saline (PBS), pH 7.2–7.6, Sigma tablets, #P4417 (or equivalent)
- β-NADH Powder (β-Nicotinamide Adenine Dinucleotide, Reduced Form) Sigma bulk catalog number N 8129, or preweighed 10-mg vials, Sigma number N6785-10VL (or BioTek PN 98233). Store the powder according to the guidelines on its packaging.

1. Prepare a PBS solution from the Sigma tablets.
2. In a beaker, mix 50 mL of the PBS solution with 10 mg of the β-NADH powder and mix thoroughly. This is the **100% Test Solution**.
3. (Optional) Read a 150-µL sample of the solution at 340 nm; it should be within 0.700 to 1.000 OD. If low, adjust up by adding more powder. Do not adjust if slightly high.

Prepare the PlateTest Procedure

1. Prepare the **75% Test Solution** by mixing 15 mL of the 100% Test Solution with 5 mL of the PBS Solution.
2. Prepare the **50% Test Solution** by mixing 10 mL of the 100% Test Solution with 10 mL of the PBS Solution.
3. Carefully pipette the three solutions into a **new** 96-well microplate:
   - 150 µL of the 100% Test Solution into all wells of columns 1 and 2
   - 150 µL of the 75% Test Solution into all wells of columns 3 and 4
   - 150 µL of the 50% Test Solution into all wells of column 5 and 6
4. Create a Gen5 experiment based on the **Synergy Neo2 Abs Test 3** protocol and read the plate.
   - Save the experiment. Refer to the instructions on page 82 to perform calculations and determine pass/fail.
   - Troubleshooting tips are provided on page 83
   - Test descriptions are provided on page 77.

Luminecence Test

Luminescence Testing, starting on page 84, describes the test method, lists the Gen5 protocol parameters, explains how to analyze the test results, and provides troubleshooting tips in the event of test failure.

Required Materials

- Harta Luminometer Reference Microplate, BTI #8030015 (which includes microplate carrier adapter BTI #8042263)
- Gen5 protocol **Synergy Neo2 LumTest_Harta.prt**, described on page 84
- LUM Upper and Lower Top filter cubes (e.g., #3 and #112, or equivalent)

Test Procedure

1. Turn on the Harta reference plate using the I/O switch on the back of the plate.
2. Check the plate's battery by pressing the test button on the back of the plate and ensuring that the test light turns on. If the light does not turn on, replace the battery.

> The test light may be difficult to see in bright light. Change your angle of view or move to a darker environment if you cannot see it.

3. Place the Harta plate adapter on the reader's carrier, then place the test plate on top of the adapter.
4. Create an experiment based on the **Synergy Neo2 LumTest_Harta** protocol, described on page 84, and read the plate.
5. When the experiment is complete, calculate and evaluate results as described under **Results Analysis** on page 85.
6. When finished, turn off the Harta reference plate to preserve battery life.

Plate Layout

	1	2	3	4	5	6	7	8	9	10	11	12
A		REF					LED7	LED8				
B												
C												
D	Buffer	Buffer	Buffer	Buffer								
E	Buffer	Buffer	Buffer	Buffer								
F	Buffer	Buffer	Buffer	Buffer								
G	Buffer	Buffer	Buffer	Buffer								
H												

Fluorescence Plate Tests

BioTek Fluorescence Test Plate on page 86 introduces the test plate and references its user guide for the test methods. Use of the test plate is offered as an alternative to conducting the fluorescence liquid tests described in the next section.

Requirements

Refer to the **Getting Started** section of the *Fluorescence Test Plate User Guide* for information on the required materials and prerequisite tasks.

Test Procedure

The **Qualification Tests** section of the *Fluorescence Test Plate User Guide* contains a procedure for cleaning the plate and then creating and running experiments based on supplied Gen5 protocols.

As described in the user guide, when each experiment is finished, Gen5 exports the measurement data to a prepared Microsoft Excel .xls file. The worksheet(s) within that file calculate results and determine pass or fail. Identify the reader-specific Gen5 protocols on the USB flash drive that came with the test plate. Use only those protocols that apply to your reader model and your organization's qualification requirements.

Fluorescence Liquid Tests

Fluorescence Liquid Tests, starting on page 88, describes the test methods, lists the Gen5 protocol parameters, explains how to analyze the test results, and provides troubleshooting tips in the event of test failure.

The tests presented in this section require specific microplates, solutions, and filters or wavelengths. Your laboratory may require a deviation from some of these tests. For example, you may wish to use a different fluorescing solution or microplate.

If deviation from the tests as presented in this section is required, the following steps should be taken the first time each test is run:

- Perform the tests exactly as described on the following pages.
- Rerun the tests using your particular plates, solutions, and so on. If results are comparable, then the results from these tests will be your baseline for future tests.
- Document your new test procedure(s), and save all test results.

Required Materials

Kits containing the microplates and solutions required by the Liquid Tests are available for purchase; see Materials for Conducting Liquid Tests on page 5.

Microplates should be perfectly clean and free from dust or bottom scratches. Use new microplates from sealed packages.

Manufacturer part numbers are subject to change.

All Tests:

- Deionized or distilled water
- Various beakers, graduated cylinders, and pipettes
- 95% ethanol (for cleaning clear-bottom plates)
- Aluminum foil
- (Optional, but recommended) 0.45-micron filter
- (Optional) Black polyethylene bag(s) to temporarily store plate(s)
- Gen5 protocols listed in the next table and described starting on page 125

For the Filter-Based Fluorescence System	
Synergy Neo2_FI_T_SF.prt	Corners, Sensitivity, and Linearity tests for top optics, using sodium fluorescein
Synergy Neo2_FI_B_SF.prt	Corners, Sensitivity, and Linearity tests for bottom optics, using sodium fluorescein
Synergy Neo2_FI_T_ MUB.prt	Alternative top optics test, using methylumbelliferone
Synergy Neo2_FI_B_ MUB.prt	Alternative bottom optics test, using methylumbelliferone
Synergy Neo2_Dual PMT_ FP.prt	Fluorescence Polarization test using dual PMT
Synergy Neo2_Single PMT_FP.prt	Fluorescence Polarization test using single PMT
Synergy Neo2_TRF.prt	Time-Resolved Fluorescence test

For the Monochromator-Based Fluorescence System	
Synergy Neo2_M_FI_T_SF.prt	Corners, Sensitivity, and Linearity tests for top optics, using sodium fluorescein
Synergy Neo2_M_FI_B_SF.prt	Corners, Sensitivity, and Linearity tests for bottom optics, using sodium fluorescein
Synergy Neo2_M_FI_T_MUB.prt	Alternative top optics test, using methylumbelliferone

Corners/Sensitivity/Linearity (FI) Tests

Manufacturer part numbers are subject to change.

The materials listed here are for use with Sodium Fluorescein. Methylumbelliferone can be used as an alternate or supplemental method for performing these tests. See page 133.

If using test kit BTI #7160010 or #7160013 (see page 4), the buffer and SF are pre-diluted.

Buffer:

- NIST-traceable Sodium Borate Reference Standard (pH 9.18) (e.g., Fisher-Scientific 1 L Sodium Borate Mfr. #159532, or equivalent), **or**
- Phosphate-Buffered Saline (PBS), pH 7.2–7.6 (e.g., Sigma tablets, Mfr. #P4417, or equivalent) and pH meter or pH indicator strips with pH range 4 to 10

- Sodium Fluorescein Powder (1 mg vial, BioTek PN 98155)
- **If testing both Top and Bottom optics:** A new, clean 96-well glass-bottom Greiner SensoPlate (Mfr. #655892), a clean Hellma Quartz 96-well titration plate (Mfr. #730.009.QG), or equivalent
- **Top optics:** A new, clean 96-well solid black microplate, such as Corning Costar #3915, or equivalent
- Filter cubes:
  - Upper Top: 3 or equivalent (dual PMT instruments)
  - Lower Top: 107 or equivalent (485/528)

Fluorescence Polarization (FP) Test

- A new, clean, 96-well solid black microplate

- The recommended test solutions are available from Invitrogen Corporation in their "FP One-Step Reference Kit" (PN P3088) or from BioTek (PN 7160014). This kit includes:
  - (Green) Polarization Reference Buffer, 15 mL
  - Green Low Polarization Reference, 4 mL
  - Green High Polarization Reference, 4 mL

The Invitrogen kit also includes two red polarization solutions; these are not used.

- Filter cubes:
  - Upper Top: 4 or equivalent (dual FP)
  - Lower Top: 61 or equivalent (FP 485/528) (dual FP)
  - 108 or equivalent (single PMT)

Time-Resolved Fluorescence (TRF) Test

- 15-mL conical-bottom, polypropylene sample tube
- Filter cubes:
  - Upper Top: 3 or equivalent (dual PMT instruments)
  - Lower Top: 112 or equivalent (360/620)
- A new, clean 96-well solid white microplate
- The recommended test solution (FluoSpheres carboxylate-modified microspheres, 0.2 µm europium luminescent, 2 µL) is available from Invitrogen Corporation (PN F20881) or from BioTek (PN 7160011)

Test Solutions

Determine which tests to run for your reader model. Prepare the necessary test solutions using the materials described on the previous pages.

Corners/Sensitivity/Linearity Tests

If using BioTek's sodium fluorescein powder (PN 98155), be sure to hold the vial upright and open it carefully; the material may be concentrated at the top. If a centrifuge is available, spin down the tube before opening.

When diluting the sodium fluorescein powder in buffer, it takes time for the powder to completely dissolve. Allow the solution to dissolve for five minutes, with intermittent vortexing, before preparing the titration dyes.

Wrap the vial containing the stock solution in foil to prevent exposure to light. Discard any open, unused solution after seven days.

1. The Sodium Borate solution does not require further preparation; proceed to step 2. If you are using PBS, prepare the solution:
   - (Optional, but recommended) Using a 0.45-micron filter, filter 200 mL of deionized or distilled water.
   - Follow the manufacturer's instructions on the PBS packaging to create 200 mL, dissolving the necessary amount of PBS into the filtered water.
   - Stir the solution (preferably using a stir table) until the PBS is completely dissolved.
   - Check the pH; it should be between 7.2 and 7.6 at 25°C.
2. Prepare the sodium fluorescein stock solution:
   - Add 2.0 mL of the buffer solution to the 1 mg Sodium Fluorescein (SF) vial. This yields a 1.3288 mM stock solution.
   - Ensure that the dye has completely dissolved and is well mixed.
3. Carefully prepare the dilutions. Label each with "SF" and the concentration:

Mix This SF Solution:	With Buffer:	To Make:	
0.53 mL of 1.3288 mM stock solution	13.47 mL	50.2 µM	
110 µL of 50.2 µM SF	13.89 mL	400 nM	
3.5 mL of 400 nM SF	10.5 mL	100 nM	
0.46 mL of 100 nM SF	13.54 mL	3.3 nM	*Corners Test*
4.24 mL of 3.3 nM SF	9.76 mL	1 nM	*Sensitivity/Linearity Tests*

Fluorescence Polarization (FP) Test

As described in **Fluorescence Polarization (FP) Tests** on page 125, the recommended test solutions are available from Invitrogen Corporation or from BioTek. They do not require additional preparation.

Time-Resolved Fluorescence (TRF) Test

As described in **Time-Resolved Fluorescence (TRF) Tests** on page 126, the recommended test solutions are available from Invitrogen Corporation or from BioTek.

- Shake the FluoSpheres container vigorously for 30 seconds prior to pipetting. Alternatively, sonicate or vortex the container.
- Mix 10 µL of FluoSpheres with 10 mL of deionized water, in a 15 mL conical-bottom, polypropylene sample tube. This yields a 20 nM equivalent suspension.
- Shake the vial vigorously for 30 seconds prior to pipetting. Alternatively, sonicate or vortex the container.
- Mix 10 µL of 20 nM suspension with 10 mL of deionized water, in a 15 mL conical-bottom, polypropylene sample tube. This yields a 20 pM equivalent suspension.
- Refrigerate any unused portions of the FluoSpheres. The temperature must be between +2°C to +6°C.

The prepared TRF plate can be kept for a maximum of seven days, if covered and stored in the dark between +2°C to +6°C.

Allow the plate to sit at room temperature for approximately 15 minutes prior to use.

Test Procedure

Top Optics

1. If you have not already done so, prepare the solutions for the tests you plan to perform. See **Fluorescence Test Solutions** on page 126.

   Refer to the pipette maps starting on page 131 for the remaining steps.

2. Perform the Corners/Sensitivity/Linearity tests using the Top optics of the filter-based fluorescence system:

- Pipette the solutions for the Corners and Sensitivity/Linearity Tests into a clean, new 96-well solid black plate.
- Create an experiment based on the **Synergy Neo2_FI_T_SF.prt** protocol.
- Perform the steps under **Determine the Optimal Read Height** to determine and set the optimal read height for the filter cube/fluid height combinations.
- Read the plate, and then save the experiment.
- If you intend to run this test in the future with the same filter cube, select **File > Save Protocol As**, and save the updated protocol with the adjusted read height. You must, however, rerun these steps if you change the filter cube used with this test.

3. Perform the Corners/Sensitivity/Linearity tests using the Top optics of the monochromator-based fluorescence system:
   - Create an experiment based on the **Synergy Neo2_M_FI_T_SF.prt** protocol. Read the plate used in step 3, and then save the experiment.
4. To test Fluorescence Polarization capability:
   - Pipette the solutions for the “FP” test into a new 96-well solid black plate.
   - Create an experiment based on the **Synergy Neo 2_Dual PMT_FP.prt** or **Synergy Neo 2_Single PMT_FP.prt** protocol. Read the plate and then save the experiment.
5. To test the Time-Resolved Fluorescence capability:
   - Pipette the solutions for the “TRF” test into a new 96-well solid white plate.
   - Create an experiment based on the **Synergy Neo2_TRF.prt** protocol. Read the plate and then save the experiment.
6. Refer to the instructions starting on page 98.
   - Troubleshooting tips are provided on page 102.
   - Test descriptions are provided on page 88.

Bottom Optics

1. If you have not already done so, prepare the solutions for the tests you plan to perform. See **Test Solutions** on page 126.

Refer to the pipette maps starting on page 131 for the remaining steps.

2. If applicable, perform the Corners/Sensitivity/Linearity tests using the Bottom optics of the filter-based fluorescence system:
   - Pipette the solutions for the Corners and Sensitivity/Linearity Tests into a clean, 96-well glass-bottom plate.
   - Create an experiment based on the **Synergy Neo2_FI_B_SF.prt** protocol.
   - Perform the steps under Determine the Optimal Read Height to determine and set the optimal read height for the filter cube/fluid height combinations.
   - Read the plate and then save the experiment.
   - If you intend to run this test in the future with the same filter cube, select **File > Save Protocol As**, and save the updated protocol with the adjusted read height. You must, however, rerun these steps if you change the filter cube used with this test.
3. Perform the Corners/Sensitivity/Linearity tests for the monochromator-based fluorescence system:
   - If you skipped step 3, pipette the solutions for the Corners and Sensitivity/Linearity Tests into a clean, new 96-well glass-bottom plate.
   - Create an experiment based on the **Synergy Neo2_M_FI_B_SF.prt** protocol. Read the plate from step 3 (or the newly created plate described above) and then save the experiment.
4. Refer to the instructions starting on page 98.
   - Troubleshooting tips are provided on page 102.
   - Test descriptions are provided on page 88.

Determine the Optimal Read Height

1. Select **Protocol > Procedure**, and open the "Sensitivity Read" step.
2. Click **Options**, clear **Automatic Gain Adjustment**, and click **OK**.
3. Click **Show advanced options** to display the Read Height information.
4. Click **Auto-Adjust**. Set the Test Well to **D7**, and click **Start Calibration**. When prompted, place the plate on the carrier and click **OK**.
5. When calibration is complete, a graph appears. Click **Select** to use the Optimal Height.
6. Click **Options** again, and select **Automatic Gain Adjustment**.
7. Record the optimal read height, then click **OK** to close the read step and return to the Procedure dialog.

8. Open the "Sensitivity Read Buffer" step. Set the read height to match the recorded value from step 7 above, then return to the Procedure dialog.
9. Open the "Corners Read" step. Set the read height to match the recorded value from step 7 above, then return to the Procedure dialog.
10. Open the "Linearity Read" step. Repeat steps 2–6 above; set the Test Well to **C1** in step 4.
11. Return to the Procedure dialog, then click **OK** to close the Procedure and return to the experiment.

Pipette Maps

Seal the plates with foil or store them in black polyethylene bags until use. When using a clear-bottom plate, if the base of the plate is touched, clean the entire base with alcohol (95% ethanol) and then wipe with a lint-free cloth. Before placing the plate in the instrument, blow the bottom of the plate with an aerosol duster.

Corners, Sensitivity, and Linearity (FI) Tests

Refer to the illustration on the next page.

Using a single-channel pipette:

- Pipette 200 µL of the **3.3 nM SF** solution into the "corner" wells.
- Pipette 200 µL of buffer into the wells surrounding the SF. (Omit if using a solid black plate or Greiner SensoPlate).
- Pipette 200 µL of the **1 nM SF** solution into well D7.
- Pipette 200 µL of the buffer solution into wells C9, D9, and E9.

Using a multichannel pipette with just four tips installed:

- Pipette 150 µL of buffer solution into wells C2–F5. Discard the tips.
- Pipette 150 µL of the **1 nM SF** solution into column 1.
- Pipette 150 µL of the 1 nM SF solution into column 2. Mix the wells using the pipette. Do not discard the tips.
- Aspirate 150 µL from column 2, and dispense into column 3. Mix the wells using the pipette. Do not discard the tips.
- Aspirate 150 µL from column 3, and dispense into column 4. Mix the wells using the pipette. Do not discard the tips.
- Aspirate 150 µL from column 4, and dispense into column 5. Mix the wells using

the pipette. Do not discard the tips.

- Aspirate 150 µL from column 5, and discard the tips.

	1	2	3	4	5	6	7	8	9	10	11	12
A	3300pM_200	3300pM_200	3300pM_200	CBUF					CBUF	3300pM_200	3300pM_200	3300pM_200
B	CBUF	CBUF	CBUF	CBUF					CBUF	CBUF	CBUF	CBUF
C	1000pM_150	500pM_150	250pM_150	125pM_150	62_5pM_150				BUF_200			
D	1000pM_150	500pM_150	250pM_150	125pM_150	62_5pM_150		1000pM_200		BUF_200			
E	1000pM_150	500pM_150	250pM_150	125pM_150	62_5pM_150				BUF_200			
F	1000pM_150	500pM_150	250pM_150	125pM_150	62_5pM_150							
G	CBUF	CBUF	CBUF	CBUF					CBUF	CBUF	CBUF	CBUF
H	3300pM_200	3300pM_200	3300pM_200	CBUF					CBUF	3300pM_200	3300pM_200	3300pM_200

Fluorescence Polarization (FP) Test

- Pipette 200 µL of the (green) polarization buffer (BUF) into wells A6–H6.
- Pipette 200 µL of the green high polarization reference (HPR) into wells A7–B7.
- Pipette 200 µL of the green low polarization reference (LPR) into wells A8–H9.

	1	2	3	4	5	6	7	8	9	10	11	12
A						BUF_FP	HPR	LPR	LPR			
B						BUF_FP	HPR	LPR	LPR			
C						BUF_FP		LPR	LPR			
D						BUF_FP		LPR	LPR			
E						BUF_FP		LPR	LPR			
F						BUF_FP		LPR	LPR			
G						BUF_FP		LPR	LPR			
H						BUF_FP		LPR	LPR			

Time-Resolved Fluorescence (TRF) Test

- Pipette 200 µL of deionized water into wells A6–C6.
- If you have not already done so, shake the vial of 20 pM europium suspension vigorously for 30 seconds prior to pipetting. Alternatively, sonicate or vortex the vial.
- Pipette 200 µL of the 20 pM europium suspension (Eu) into well A8.

	1	2	3	4	5	6	7	8	9	10	11	12
A						BUF		Eu				
B						BUF						
C						BUF						
D												
E												
F												
G												
H												

Alternate/Supplemental Tests Using Methylumbelliferone (MUB)

As an alternative to using Sodium Fluorescein, Methylumbelliferone (“MUB”) can be used to test the top and bottom optics for filter-based fluorescence systems and the top optics for monochromator-based fluorescence systems.

Required Materials

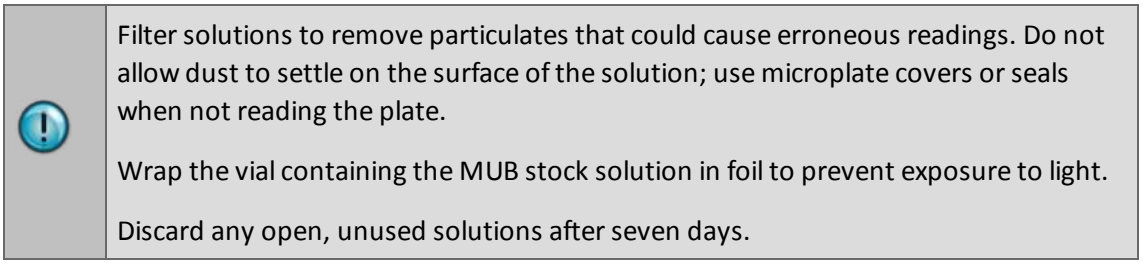
Microplates should be perfectly clean and free from dust or bottom scratches. Use new microplates from sealed packages.

Manufacturer part numbers are subject to change over time.

BioTek offers a liquid test kit (PN 7160012) containing the microplates and solutions used in the MUB fluorescence liquid tests for the Top optics. If also testing the Bottom optics (filter-based system), you will need an additional microplate (see below).

- Methylumbelliferone ("MUB") (10-mg vial, BioTek PN 98156)
- Carbonate-Bicarbonate buffer ("CBB") capsules (BioTek PN 98158)
- 100% methanol (BioTek PN 98161)
- **Top optics:** A new, clean 96-well solid black plate microplate, such as Corning Costar #3915 or equivalent. The same plate is used to test both filter- and monochromator-based systems.
- **Bottom optics:** A new, clean 96-well glass-bottom Greiner SensoPlate (Mfr. #655892), a clean Hellma Quartz 96-well titration plate (Mfr. #730.009.QG), or equivalent
- Filter cube 107 or equivalent (360/460)
- Deionized or distilled water
- Various beakers, graduated cylinders, and pipettes
- 95% ethanol (for cleaning clear-bottom plates)
- Aluminum foil
- (Optional, but recommended) 0.45-micron filter
- (Optional) Black polyethylene bag(s) to temporarily store plate(s)
- Gen5 protocols described on page 133:
  - **Synergy Neo 2_FI_T_MUB.prt** tests the top filter-based fluorescence system
  - **Synergy Neo 2_FI_B_MUB.prt** tests the bottom filter-based fluorescence system
  - **Synergy Neo 2_M_FI_T_MUB.prt** tests the top optics of the monochromator-based fluorescence system

Test Solutions



Filter solutions to remove particulates that could cause erroneous readings. Do not allow dust to settle on the surface of the solution; use microplate covers or seals when not reading the plate.

Wrap the vial containing the MUB stock solution in foil to prevent exposure to light.

Discard any open, unused solutions after seven days.

1. Prepare the buffer (CBB) solution:
   - (Optional, but recommended) Using a 0.45-micron filter, filter 200 mL of deionized or distilled water.

- Open and dissolve the contents of two CBB capsules (do not dissolve the outer gelatin capsule) into 200 mL of the water.
- Stir the solution (preferably using a stir table) until the CBB is completely dissolved.

2. Prepare the MUB stock solution:
   - Add 1 mL of 100% methanol to the 10 mg vial of MUB.
   - Make sure all of the dye has completely dissolved and is well mixed. This yields a 10 mg/mL stock solution.
   - Wrap the solution in aluminum foil to prevent exposure to light.
3. Prepare the dilutions. Label each with "MUB" and the concentration.

Mix This MUB Solution:	With:	To Make:
0.5 mL of 10 mg/mL stock solution	4.5 mL of 100% methanol	1 mg/mL
0.88 mL of 1 mg/mL solution	4.12 mL of CBB	176 µg/mL
0.1 mL of 176 µg /mL solution	9.9 mL of CBB	1.76 µg /mL
0.5 mL of 1.76 µg /mL solution	4.5 mL of CBB	176 ng/mL
1 mL of 176 ng/mL solution	9 mL of CBB	17.6 ng/mL (100 nM)

Test Procedure

1. If you have not already done so, prepare the test solutions. See page 134.

> Refer to the pipette map on the next page for the remaining steps.

2. Perform the Sensitivity and Linearity tests using the Top optics of the filter-based fluorescence system:
   - Pipette the solutions into a clean, 96-well plate.
   - Create an experiment based on **Synergy Neo 2 FI_T_MUB.prt**.
   - Perform the steps under **Determine the Optimal Read Height** to determine and set the optimal read height for the filter cube/fluid height combination.
   - Read the plate, and save the experiment.
   - If you intend to run this test in the future **with the same filter cube**, select **File > Save Protocol As**, and save the updated protocol with the

adjusted read height. You must, however, rerun these steps if you change the filter cube used with this test.

3. Perform the Sensitivity and Linearity tests for the monochromator-based fluorescence system:
   - Create an experiment based on **Synergy Neo 2_M_FI_T_MUB.prt**.
   - Read the plate, and save the experiment
4. If applicable, perform the Sensitivity and Linearity tests using the Bottom optics of the filter-based fluorescence system:
   - Create an experiment based on **Synergy Neo 2_FI_B_MUB.prt**.
   - Perform the steps under Determine the Optimal Read Height to determine and set the optimal read height for the filter cube/fluid height combination.
   - Read the plate, and then save the experiment.
   - (Optional) Save the updated protocol with the adjusted read height.
5. Refer to the instructions starting on page 133
   - Troubleshooting tips are provided on page 102.
   - Test descriptions are provided on page 128.

Determine the Optimal Read Height

1. Select **Protocol > Procedure**, and open the "Sensitivity Read" step.
2. Click **Options**, clear **Automatic Gain Adjustment**, and click **OK**.
3. Click **Show advanced options** to display the Read Height information.
4. Click **Auto-Adjust**. Set the Test Well to **D7**, and click **Start Calibration**. When prompted, place the plate on the carrier and click **OK**.
5. When calibration is complete, a graph appears. Click **Select** to use the Optimal Height.
6. Click **Options** again, and select **Automatic Gain Adjustment**.
7. Record the optimal read height, then click **OK** to close the read step and return to the Procedure dialog.
8. Open the "Sensitivity Read Buffer" step. Set the read height to match the recorded value from step 7 above, then return to the Procedure dialog.
9. Open the "Linearity Read" step. Repeat steps 2–6 above; set the Test Well to **C1** in step 4.
10. Return to the Procedure dialog, then click **OK** to close the Procedure and return to the experiment.

Pipette Map

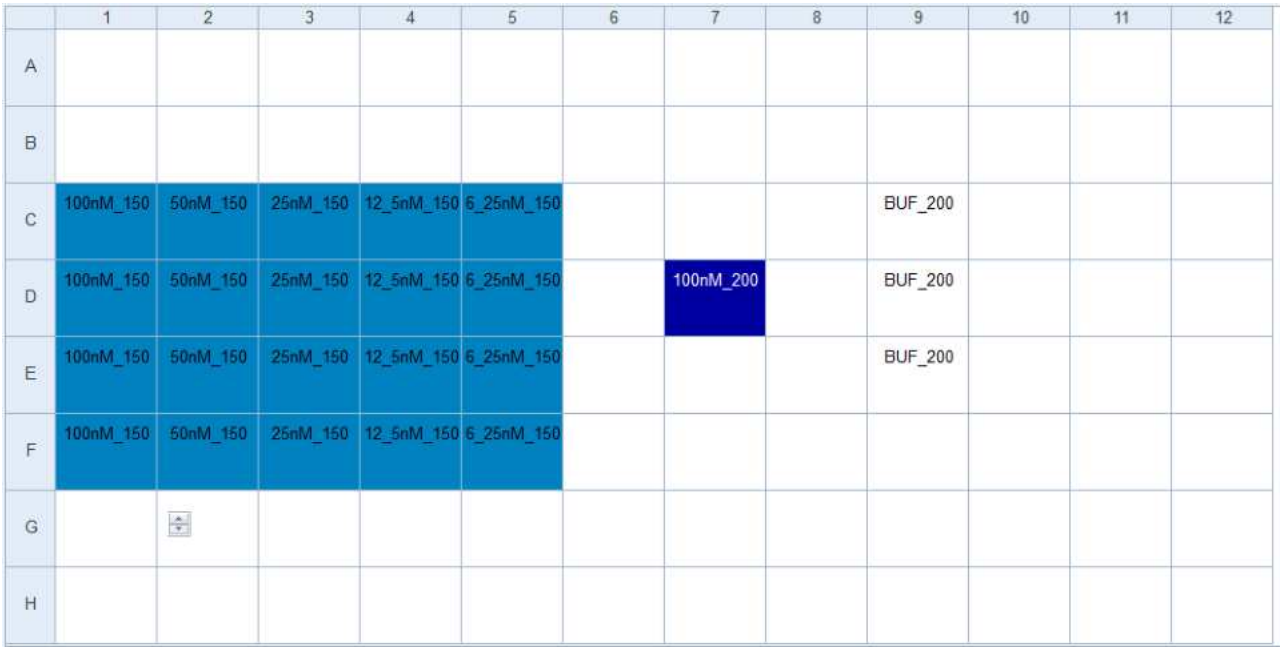
Seal the plate with foil or store it in a black polyethylene bag until use. When using a clear-bottom plate, if the base of the plate is touched, clean the entire base with alcohol (95% ethanol) and then wipe with a lint-free cloth. Before placing a plate in the instrument, blow the bottom of the plate with an aerosol duster.

Using a single-channel pipette:

- Pipette 200 µL of 17.6 ng/mL (100 nM) MUB solution into well D7.
- Pipette 200 µL of buffer into wells C9, D9, and E9.

Using a multichannel pipette with just four tips installed:

- Pipette 150 µL of buffer into columns 2–5 (**not column 1**). Discard the tips.
- Pipette 150 µL of the 17.6 ng/mL (100 nM) solution into column 1. Discard the tips.
- Pipette 150 µL of the 17.6 ng/mL (100 nM) solution into column 2. Do not discard the tips.
- Aspirate 150 µL from column 2 and dispense it into column 3. Mix the wells using the pipette. Do not discard the tips.
- Aspirate 150 µL from column 3 and dispense it into column 4. Mix the wells using the pipette. Do not discard the tips.
- Aspirate 150 µL from column 4 and dispense it into column 5. Mix the wells using the pipette. Do not discard the tips.
- Aspirate 150 µL from column 5. Discard the tips.



Injection System Tests

Injection System Testing, starting on page 104, describes the test methods, lists the Gen5 protocol parameters, explains how to analyze the test results, and provides troubleshooting tips in the event of test failure.

Required Materials

Manufacturer part numbers are subject to change over time.

- Absorbance reader with capability of reading at 405, 630, and 750 nm. The reader must have an accuracy specification of ± 1.0% ± 0.010 OD or better and a repeatability specification of ± 1.0% ± 0.005 OD or better.

  The Synergy Neo2 may be used if it has passed the Absorbance Plate Test or Absorbance Liquid Test 2, and Absorbance Liquid Test 1.

- Microplate shaker (if the absorbance reader does not support shaking)
- Precision balance with capacity of 100 g minimum and readability of 0.001 g
- 50–200 µL hand pipette and disposable tips
- Deionized water

- Supply bottles
- 250-mL beaker
- New 96-well, clear, flat-bottom microplates
- BioTek's Green Test Dye Solution (PN 7773003) undiluted, or one of the alternate test solutions listed in the next section
- 100-mL graduated cylinder and 10-mL pipettes (if not using BioTek's Green Test Dye Solution)
- Gen5 software installed on the host PC
- Gen5 protocols listed below (as applicable to your reader model) and described in detail under Gen5 Parameters, starting on page 104

  Synergy Neo2 Disp 1 Test.prt
  Synergy Neo2 Disp 2 Test.prt

Alternate Test Solutions

If you do not have BioTek's Green Test Dye Solution (PN 7773003), prepare a dye solution using one of the following methods:

80 µL of test solution with 150 µL of deionized water should read between 1.300 and 1.700 OD at 405/750 nm. The solutions should be at room temperature.

Using BioTek's Blue and Yellow Concentrate Dye Solutions:

Item	Quantity
Concentrate Blue Dye Solution (PN 7773001, 125 mL)	4.0 mL
QC (Yellow) Solution (PN 7120782, 125 mL)	5.0 mL
Deionized water	90.0 mL

Using FD&C Blue and Yellow Dye Powder:

Item	Quantity per Liter
FD&C Blue No. 1	0.200 grams
FD&C Yellow No. 5	0.092 grams
Tween 20	1.0 mL
Sodium Azide N_3Na	0.100 gram
Deionized water	Make to 1 liter

Test Procedure

1. Prime both dispensers with 4000 µL of deionized or distilled water.
2. Remove the inlet tubes from the supply bottles. Prime both dispensers with the Volume set to 2000 µL. This prevents the water from diluting the dye.
3. Fill a beaker with at least 20 mL of the green dye solution. Prime both dispensers with 2000 µL of the solution. When finished, remove the priming plate from the carrier.
4. In Gen5, create an experiment based on **Synergy Neo2_Disp 1 Test.prt**.
5. Place a new 96-well microplate on the balance and tare the balance.
6. Place the plate on the microplate carrier.

Running a dispense procedure without placing a plate in the reader will result in contamination of the reader from spilled liquid.

When each dispense step is finished, you will weigh the plate, record the weight, tare the balance with the plate on it, and then place the plate back on the carrier for the next step.

7. Initiate a plate read. Gen5 prompts you to empty the tip priming trough.
8. When ready, click **OK** at the Load Plate dialog to begin the experiment. The sequence is as follows:
   a. Dispense 80 µL/well to columns 1–4.
   b. Remove the plate and weigh it. Record the weight and tare the balance.
   c. Place the plate on the carrier and dispense 20 µL/well to columns 5–8.
   d. Remove the plate and weigh it. Record the weight and tare the balance.

e. Place the plate on the carrier and dispense 5 µL/well to columns 9–12.

f. Remove the plate and weigh it. Record the weight.

g. Manually pipette 150 µL of deionized or distilled water into all 12 columns, on top of the green test dye solution.

h. Place the plate on the carrier for a 30-second shake, the "80 µL" read at 405/750 nm, and the "20 and 5 µL" read at 630/750 nm.

9. When finished, select **File > Save As**. Enter an identifying file name and click **Save**.
10. Remove the plate from the carrier and set it aside.
11. Repeat steps 4–9 using **Synergy Neo2_Disp 2 Test.prt**.
12. When the tests are complete:
    - Refer to the instructions on page 105 to perform calculations and determine pass/fail.
    - Test descriptions are provided on page 104

When all tests are complete, prime both dispensers with at least 5000 µL of deionized water to flush out the green dye solution.

For your convenience, worksheets are included at the end of this chapter for recording the dispense weights, Delta OD values, calculations, and pass/fail.

Gen5 Test Protocols

1. Select **System > Instrument Configuration**, and add/configure the Synergy Neo2 (if it is not already there).
2. Create a new protocol.

To edit a protocol category, double-click its "branch" in the tree.

3. Perform the steps in the following three sections to define the Procedure, customize the Plate Layout, and add Data Reduction steps, to test Dispenser #1.
4. When finished, select **File > Save As** and save the file as **Synergy Neo2_Disp 1 Test.prt**.
5. Repeat steps 2–4 above to create **Synergy Neo2_Disp 2 Test.prt** to test Dispenser 2.

Define the Procedure

In brief, the protocol's procedure follows the sequence below. After each Dispense step, the plate is ejected to allow the operator to weigh it and then tare the balance.

- Dispense 80 µL dye to columns 1–4
- Dispense 20 µL dye to columns 5–8
- Dispense 5 µL dye to columns 9–12
- Shake the plate for 30 seconds
- Read columns 1–4 at 405/750 nm and calculate the Delta OD
- Read columns 5–12 at 630/750 nm and calculate the Delta OD

The detailed procedure is described on the next page. To add a step to the procedure, click the appropriate button on the left side of the Procedure dialog and define the required parameters.

The comments suggested for use with the Plate Out/In steps are optional, but they may be useful for the person running the test. When the Plate Out/In step is executed, Gen5 displays its comment in a message box.

Gen5 Procedure Steps

Step Type	Details
1. Dispense	Dispenser <select 1 or 2, depending on the protocol> Dispense to wells A1..H4 Tip prime before this dispense step, 20 µL Dispense 80 µL at rate 275 µL/sec
2. Plate Out,In	Suggested comment: Weigh the plate (80 µL test). RECORD the weight, TARE the balance. Place the plate back on the carrier. Click **OK** to continue.
3. Dispense	Dispenser <select 1 or 2, depending on the protocol> Dispense to wells A5..H8 Tip prime before this dispense step, 20 µL Dispense 20 µL at rate 250 µL/sec
4. Plate Out,In	Suggested comment: Weigh the plate (20 µL test). RECORD the weight and TARE the balance. Place the plate back on the carrier. Click **OK** to continue.

Gen5 Procedure Steps

Step Type	Details
5. Dispense	Dispenser <select 1 or 2, depending on the protocol> Dispense to wells A9..H12 Tip prime before this dispense step, 5 µL Dispense 5 µL at rate 225 µL/sec
6. Plate Out,In	Suggested comment: Weigh the plate (5 µL test). RECORD the weight. PIPETTE 150 µL/well of DI water into all 12 columns. Place the plate back on the carrier. Click **OK** to perform the Read steps.
7. Shake	Orbital at 425 cpm (3 mm) for 30 seconds.
8. Read	Step label: “80 ul Read_Disp 1” (or _Disp 2) Wells: A1..H4 Detection Method: Absorbance Read Type: Endpoint Read Speed: Normal Two Wavelengths: 405 and 750 nm
9. Read	Step label: “20 and 5 ul Read_Disp 1” (or _Disp 2) Wells: A5..H12 Detection Method: Absorbance Read Type: Endpoint Read Speed: Normal Two Wavelengths: 630 and 750 nm

Customize the Plate Layout (Optional)

The results analysis worksheet at the end of this chapter requires the calculation of the Standard Deviation, Mean, and % CV of the ODs read for each dispense volume in each plate (six sets of calculations). By identifying the wells by their dispense volumes in the Plate Layout, Gen5 will calculate these values for you.

1. In the protocol, open the Plate Layout dialog.
2. Set the Type set to **Assay Control** and define three control types: Disp_80, Disp_20, and Disp_5.
3. In the Plate Layout, select **Disp_80** and highlight wells A1 to H4.
4. Select **Disp_20** and highlight wells A5 to H8.
5. Select **Disp_5** and highlight wells A9 to H12.
6. Click **OK** to save the changes and close the dialog.

After running the experiment, view the Statistics for each Delta OD Data Set to view the calculations

Add Data Reduction Steps

Each Read step is performed using two wavelengths, so you will create two data reduction steps to calculate the Delta OD values.

1. In the protocol, open the Data Reduction dialog and click **Custom**. **Transformation**.
2. Click **Select multiple data sets** and then select **DS2**.
3. Set the Data In for DS1 to the 80 µL Read step at 405 nm.
4. Set the Data In for DS2 to the 80 µL Read step at 750 nm.
5. Click **OK** to return to the Transformation dialog.
6. In the New Data Set Name field, type an identifying name such as **Delta OD 80 ul_ Disp 1**.
7. Clear **Use single formula for all wells**.
8. In the Current Formula field, type DS1–DS2 and then highlight wells A1 to H4 to assign the formula.
9. Click **OK** to add the transformation to the Data Reduction list.
10. Create another Transformation similar to the above, with these characteristics:
    - DS1 set to the 20 and 5 µL Read step at 630 nm
    - DS2 set to the 20 and 5 µL Read step at 750 nm
    - New Data Set Name resembling **Delta OD 20 and 5 uL_Disp 1**
    - Remember to clear **Use single formula for all wells**
    - Formula DS1–DS2 applied to wells A5 to H12

11. When you are finished, the Data Reduction Steps list shows two Delta OD transformations:
12. Click **OK** to close the Data Reduction dialog.

Alpha Detection Testing

Alpha Detection Test, starting on page 106, describes the test methods, lists the Gen5 protocol parameters, explains how to analyze the test results, and provides troubleshooting tips in the event of test failure.

1. Pipette 100 µL of PBS in wells A1–A12, B1, B3–B4, B6–B7, B9–B10, B12, C1–C12, and G1–H12 (see pipette map, "BUF" wells).
2. Pipette 100 µL of 20 µg/mL Omnibead suspension into wells B2, B5, B8, and B11 (see pipette map, "20 µg/mL OMB" wells).

Allow the plate to sit at room temperature for approximately 15 minutes prior to use.

3. Create an experiment based on the **Synergy Neo2 AlphaTest_Crosstalk.prt** protocol. Read the plate and then save the experiment.
4. When the test is complete:
   - Refer to the instructions on page 106 to perform calculations and determine pass/fail.
   - Test descriptions are provided on page 106.

Required Materials

Manufacturer part numbers are subject to change over time.

- Recommended test solution, Alphascreen Omnibead Assay kit, available from PerkinElmer (PN 6760626)
- Buffer: Phosphate-Buffered Saline (PBS), pH 7.2–7.6 (e.g., Sigma tablets, Mfg. #P4417 or equivalent)
- Clean 96-well solid white microplate, Costar #3912
- 15-mL conical-bottom, polypropylene sample tube
- Filter cubes:

- Dual PMT system: 2 or equivalent (Alpha Top)
- 1 or equivalent (Alpha Bottom)

- Gen5 protocol **Synergy Neo2_AlphaTest_Crosstalk.prt**, described in detail on page 106

Test Solutions

Alphascreen Omnibeads are light sensitive. All tests should be performed under subdued laboratory lighting of less than 100 lux.

1. Prepare the PBS buffer solution:
   a. (Optional, but recommended) Using a 0.45-micron filter, filter 200 mL of deionized or distilled water.
   b. Follow the manufacturer's instructions on the PBS packaging to create 200 mL, dissolving the necessary amount of PBS into the filtered water.
   c. Stir the solution (preferably using a stir table) until the PBS is completely dissolved.
   d. Check the pH; it should be between 7.2 and 7.6 at 25°C.
2. Prepare the Omnibead suspension:
   a. Shake the container of 5 mg/mL Omnibead suspension vigorously for 30 seconds prior to pipetting. Alternatively, sonicate or vortex the container.
   b. Mix 20 µL of 5 mg/mL Omnibead suspension with 4.98 mL of PBS in a 15 mL conical bottom, polypropylene sample tube. This yields a 20 µg/mL Omnibead suspension.
   c. Refrigerate any unused portions of the Omnibeads. The temperature must be between +2°C and +6°C.

Pipette Map

	1	2	3	4	5	6	7	8	9	10	11	12
A	BUF	BUF	BUF	BUF	BUF	BUF	BUF	BUF	BUF	BUF	BUF	BUF
B	BUF	OMB1	BUF	BUF	OMB1	BUF	BUF	OMB1	BUF	BUF	OMB1	BUF
C	BUF	BUF	BUF	BUF	BUF	BUF	BUF	BUF	BUF	BUF	BUF	BUF
D												
E												
F												
G	BUF	BUF	BUF	BUF	BUF	BUF	BUF	BUF	BUF	BUF	BUF	BUF
H	BUF	BUF	BUF	BUF	BUF	BUF	BUF	BUF	BUF	BUF	BUF	BUF

Test Procedure

1. Pipette 100 µL of PBS in wells A1–A12, B1, B3–B4, B6–B7, B9–B10, B12, C1–C12, and G1–H12 (see pipette map, “BUF“ wells).
2. Pipette 100 µL of 20 µg/mL Omnibead suspension into wells B2, B5, B8, and B11 (see pipette map, “20 µg/mL OMB” wells).
3. Allow the plate to sit at room temperature for approximately 15 minutes prior to use.
4. Create an experiment based on the **Synergy Neo2 AlphaTest_Crosstalk.prt** protocol. Read the plate and then save the experiment.
5. Open the Plate menu and export the data to the embedded Power Export spreadsheet. The spreadsheet reports pass or fail for the test performed.
6. Print the spreadsheet and store it with your test records.

Synergy Neo 2 Dispense Accuracy & Precision Tests - Dispenser #1

80 µL Dispense Delta ODs @405/750 nm

	1	2	3	4	
A					A
B					B
C					C
D					D
E					E
F					F
G					G
H					H

80 µL weight: _____ g

Expected weight: 2.5600 g

Accuracy % Error: _____ %

Must be <= 2.0% ☐ P ☐ F

Standard Deviation: _____

Mean: _____

%CV: _____ %

Must be <= 2.0% ☐ P ☐ F

20 µL Dispense Delta ODs @630/750 nm

	5	6	7	8
A				
B				
C				
D				
E				
F				
G				
H				

20 µL weight: _____ g

Expected weight: 0.6400 g

Accuracy % Error: _____ %

Must be <= 5.0% ☐ P ☐ F

Standard Deviation: _____

Mean: _____

%CV: _____ %

Must be <= 7.0% ☐ P ☐ F

5 µL Dispense Delta ODs @630/750 nm

	9	10	11	12	
A					A
B					B
C					C
D					D
E					E
F					F
G					G
H					H

5 µL weight: _____ g

Expected weight: 0.1600 g

Accuracy % Error: _____ %

Must be <= 20.0% ☐ P ☐ F

Standard Deviation: _____

Mean: _____

%CV: _____ %

Must be <= 10.0% ☐ P ☐ F

Reader Model: _____

Reader S/N: _____

Reading Date: _____

Comments:

Tested By: _____

Signature: _____

Reviewed/ Approved By: _____

Signature: _____

Synergy Neo 2 Dispense Accuracy & Precision Tests - Dispenser #2

	80 µL Dispense Delta ODs @405/750 nm			
	1	2	3	4
A				
B				
C				
D				
E				
F				
G				
H				

80 µL weight: _____ g

Expected weight: 2.5600 g

Accuracy % Error: _____ %

Must be <= 2.0% ☐ P ☐ F

Standard Deviation: _____

Mean: _____

%CV: _____ %

Must be <= 2.0% ☐ P ☐ F

	20 µL Dispense Delta ODs @630/750 nm			
	5	6	7	8
A				
B				
C				
D				
E				
F				
G				
H				

20 µL weight: _____ g

Expected weight: 0.6400 g

Accuracy % Error: _____ %

Must be <= 5.0% ☐ P ☐ F

Standard Deviation: _____

Mean: _____

%CV: _____ %

Must be <= 7.0% ☐ P ☐ F

	5 µL Dispense Delta ODs @630/750 nm				
	9	10	11	12	
A					A
B					B
C					C
D					D
E					E
F					F
G					G
H					H

5 µL weight: _____ g

Expected weight: 0.1600 g

Accuracy % Error: _____ %

Must be <= 20.0% ☐ P ☐ F

Standard Deviation: _____

Mean: _____

%CV: _____ %

Must be <= 10.0% ☐ P ☐ F

Reader Model: _____

Reader S/N: _____

Reading Date: _____

Comments:

Tested By: _____

Signature: _____

Reviewed/
Approved By: _____

Signature: _____

Appendix A

Specifications

This appendix contains BioTek's published specifications for the Synergy Neo2.

General Specifications

Microplates	
The Synergy Neo2 accommodates standard 6-, 12-, 24-, 48-, 96-, 384-, and 1536-well microplates with 128 x 86 mm geometry and the Take3 and Take3 Trio Micro-Volume Plates. Maximum Plate Height: 0.89"	
Hardware and Environmental	
Light Source	
Absorbance, Fluorescence (FI), monochromator-based:	Xenon flash light source, 20W maximum average power (not user-changeable)
Fluorescence (FI/FP), filter-based:	Xenon flash light source, 5W maximum average power (not user-changeable)
TRF (filter-based):	Xenon flash light source, 5W maximum average power (not user-changeable)
Dimensions	20.7" D x 15.5" W x 16.1" H 52.5 cm D x 39 cm W x 41 cm H
Weight:	With all modules installed, without power supply or dispense module attached, < 85 lbs. (38.6 kg)
Environment:	Power-up temperature 18º to 30ºC Operational temperature 18º to 40ºC
Humidity:	10% to 85% relative humidity (non-condensing)
Power Supply:	24-volt external power supply compatible with 100–240 V~; +/- 10% @50–60 Hz
Power Consumption:	250W maximum
Incubation:	**Alphascreen only:** Temperature control range from 3ºC over ambient to 30ºC. **All other modes:** Temperature control range from 4ºC over ambient to 65ºC. Temperature variation ± 0.5ºC across the plate @ 37ºC, tested with Innovative Instruments, Inc. temperature test plate

Dispense/Read Specifications

Maximum Delay between End of Dispense and Beginning of Read 96/384-Well Plates, Default Probe Heights	
Bottom Mono Fluorescence	T < 1.0 second
Luminescence	T < 1.0 second
Top Filter Fluorescence	T < 1.0 second
Top Mono Fluorescence	T < 1.0 second
Absorbance	T < 1.0 second
Bottom Filter Fluorescence	T < 1.0 second

Dispense/Read, for Models with the Dual-Reagent Dispense Module	
Plate Type	Both injectors dispense to standard height 6-, 12-, 24-, 48-, 96-, and 384-well microplates.
Detection Method	Absorbance, Fluorescence (FI, FP, TRF), Luminescence
Volume Range	5–1000 µL with a 5–20 µL tip prime
Reagent Dead Volume	< 1100 µL, with dead volume recovery function (back flush)
Injection Speeds	225, 250, 275, 300 µL/second
Accuracy	± 1µL or 2.0%, whichever is greater
Precision	< 2.0% for volumes of 50–200 µL < 4.0% for volumes of 25–49 µL < 7.0% for volumes of 10–24 µL < 10.0% for volumes of 5–9 µL

Absorbance Specifications

Optics	
Wavelength Range	230 to 999 nm
Wavelength Bandpass	< 4 nm (230–285 nm), < 8 nm (> 285 nm)
Measurement Range	0.000 to 4.000 OD
Resolution	0.0001 OD
Increment	1 nm
Wavelength Accuracy	± 2 nm
Wavelength Precision	± 0.2 nm
Minimum kinetic interval (450 nm)	< 12 seconds, sweep mode, 384-well microplate

Plate In/Plate Out Speed
< 20 seconds, 450 nm, sweep mode, 384-well microplate

Accuracy, Linearity, Repeatability
Specifications apply from 250–999 nm, 200 µL (96-well microplates) and 80 µL (384-well microplates).
Accuracy (tested with certified neutral density glass)
96-well plate, normal read speed 0–2 OD: +/-1% +/-0.010 OD, delay after plate movement = 100 ms 2–2.5 OD: +/-3% +/-0.010 OD, delay after plate movement = 100 ms
384-well plate, normal read speed 0–2 OD: +/-2% +/-0.010 OD, delay after plate movement = 100 ms 2–2.5 OD: +/-5% +/-0.010 OD, delay after plate movement = 100 ms
96-well and 384-well plate, sweep read speed 0–1 OD: +/-2% +/-0.010 OD
Linearity (by liquid dilution)
96-well plate, normal read speed 0–2 OD: +/-1% +/-0.010 OD, delay after plate movement = 100 ms 2–2.5 OD: +/-3% +/-0.010 OD, delay after plate movement = 100 ms
384-well plate, normal read speed 0–2 OD: +/-2% +/-0.010 OD, delay after plate movement = 100 ms 2–2.5 OD: +/-5% +/-0.010 OD, delay after plate movement = 100 ms
96-well and 384-well plate, sweep read speed 0–1 OD: +/-2% +/-0.010 OD
Repeatability (tested with certified neutral density glass/measured by one standard deviation: 8 measurements per data point)
96-well and 384-well plate, normal read speed 0–2 OD: +/-1% +/-0.005 OD, delay after plate movement = 100 ms 2–2.5 OD: +/-3% +/-0.005 OD, delay after plate movement = 100 ms
96-well and 384-well plate, sweep read speed 0–1 OD: +/-2% +/-0.010 OD

Take3 Plate	
Detection Limit, 260 nm dsDNA	< 5 ng/µL

Fluorescence Specifications (Mono-Based)

The Synergy Neo2 measures fluorescence with monochromators from the top and bottom of 6- to 1536-well plates.

Monochromator-Based Fluorescence	
Excitation range	250–900 nm with red-shifted PMT
Emission range	250–900 nm with red-shifted PMT; 300 nm minimum with EM scan.
Bandpass	Excitation and Emission: Variable, from 3 nm–50 nm (wavelength dependent) in 1 nm increments

Optical Probes	
Top position	2.00 mm diameter fixed
Bottom position	2.00 mm diameter fixed

Plate In/Plate Out Speed
< 20 seconds for filter set, sweep mode, 384-well microplate

Sensitivity	
96-/384-well plates	*Sodium Fluorescein in phosphate buffered saline (PBS)* DL <= 20 pM top or bottom read, 2 pM typical Excitation 485 nm, Emission 528 nm *Methylumbelliferone (MUB) in carbonate-bicarbonate buffer (CBB)* DL <= 0.16 ng/mL (0.91 nM) top read Excitation 360 nm, Emission 460 nm *Propidium Iodide (PI) in PBS* DL <= 62.5 ng/mL bottom read Excitation 485 nm, Emission 645 nm

Fluorescence Specifications (Filter-Based)

The Synergy Neo2 measures fluorescence with filters from the top and bottom of 6- to 1536-well plates.

Optical Probes	
Top position	2.6-mm diameter fixed
Bottom position	2.6-mm diameter fixed

Top Single PMT

Plate In/Plate Out Speed
< 20 seconds for filter set, sweep mode, 384-well microplate

Sensitivity	
96-/384-well plates	DL < 5 pM (< 1 pM typical) solution of Sodium Fluorescein in PBS Excitation 485/20, Emission 528/20, 510 nm mirror DL ≤ 0.16 ng/mL (0.91 nM) solution of Methylumbelliferone in CBB, Excitation 360/40, Emission 460/40, 400 nm mirror

Time-Resolved Fluorescence	
96-/384-well plates	DL Europium ≤ 250 fM (100 fM typical) Excitation 360/40 nm, Emission 620/40 nm 400 nm mirror
Integration Interval	20 to 2000 µs
Delay	0 to 2000 µs
Granularity	1-µs step

Top Double PMT

Plate In/Plate Out Speed
< 20 seconds for filter set, sweep mode, 384-well microplate

Fluorescence Polarization	
96-/384-well plate	< 5 mP standard deviation at 1 nM Sodium Fluorescein (1 mP typical) Excitation 485/20 nm, Emission 528/20 nm, 510 nm mirror

Bottom Optics

Sensitivity	
96-/384-well plates	DL < 5 pM (< 2 pM typical) solution of Sodium Fluorescein in PBS Excitation 485/20, Emission 528/20, 510 nm mirror DL ≤ 0.16 ng/mL (0.91 nM) solution of Methylumbelliferone in CBB, Excitation 360/40, Emission 460/40, 400 nm mirror

Luminescence Specifications

The Synergy Neo2 measures luminescence from the top of 6- to 384-well plates. The following requirements apply to 96-well plates with 200 µL/well, at room temperature.

Production testing is performed using a Harta plate.

Luminescence	
96-well plate, standard low-noise PMT	≤ 50 amol/well, 10 amol typical
Integration Time	10 seconds
Blank Wells	16
Cross-talk	< 0.1% in 96-well plate < 0.25% in low-volume 384-well plate

Alpha Detection Specifications

Alpha Laser	
Laser Output Power	100 mW ± 10%

Appendix B

Error Codes

This appendix lists and describes Synergy Neo2 error codes that may appear in Gen5.

Error Codes Overview

When a problem occurs during operation with the Synergy Neo2, an error code appears in Gen5. Error codes typically contain four characters, such as “4168,” and in most cases are accompanied by descriptive text, such as “PMT overload error.” With many errors, the instrument will beep repeatedly; press the carrier eject button to stop this alarm.

Some problems can be solved easily, such as “2B0A: Priming plate not detected” (place a priming plate on the carrier). Some problems can be solved only by trained BioTek service personnel. This appendix lists the most common and easily resolved error codes that you may encounter.

Error codes beginning with “A” (e.g., A100) indicate conditions that require immediate attention. If this type of code appears, turn the instrument off and on. If the System Test does not conclude successfully, record the error code and contact BioTek’s Technical Assistance Center.

If an error code appears in Gen5, you may want to run a System Test for diagnostic purposes. In Gen5, select **System > Diagnostics > Run System Test**.

If an error message appears while an experiment is in process and after having received measurement data, it is your responsibility to determine if the data is valid.

Contact Info: BioTek Service/TAC

Use this appendix to diagnose problems and solve them if possible. If you need further assistance, contact BioTek’s Technical Assistance Center.

Phone: 800-242-4685 (toll free in the U.S.)

802-655-4740 (outside the U.S.)

Fax: 802-654-0638

E-Mail: tac@biotek.com

For errors that are displayed during operation of the Synergy Neo2 with the stacker or the BioStack 4, refer to the stacker's or Biostack's operator's manuals.

Error Codes

This table lists the most common and easily resolved error codes that you may encounter. If an error code appears in Gen5, look for it here. If you find the code, follow the suggestions provided for solving the problem. If you cannot find the code or if you are unable to solve

the problem, please contact BioTek's Technical Assistance Center. The Gen5 Help system also provides troubleshooting tips.

Code	Description and Possible Remedy
2353	**Expected plug/hole/filter not found in filter cube** This error indicates that the filter cube is not installed and is required for the read. Install the filter cube or check that it is installed correctly.
2700	**Error attempting to run the barcode scanner SET command** A response returned from the scanner is invalid.
2701	**Error attempting to run the barcode scanner SET command** The command message is calling out an invalid barcode location. Valid numbers are 1–4.
2702	**Error attempting to get barcode scanner information regarding one of the barcode types** The barcode type returned by the scanner is not one of those expected.
2703	**Barcode type is not supported** One of the four barcode types is not supported by the scanner.
2704	**Error disabling start/stop character transmission** While attempting to tell the scanner to disable the transmission of start and stop characters along with the barcode value for the Codebar barcode type, an error occurred.
2B0A	**Priming plate not detected**
2B0x	**Dispenser syringe 1 or 2 (respectively) did not home** x=1–3 Generally, this error indicates the syringe was not properly installed. Make sure the syringe's thumbscrews are properly threaded. (Refer to the **Installation** chapter for instructions.) Restart the reader.
2B04	**Dispenser syringe 1 or 2 (respectively) failed position verify** Generally, this error indicates the syringe was not properly installed. Make sure the syringe's thumbscrews are properly threaded. Restart the reader. (Refer to the **Installation** chapter for instructions.)

Code	Description and Possible Remedy
37x0/47x0 38x0/48x0 39xy/49xy	**Noise Test Errors** **Offset Test Errors** **Dark Range Errors** x=0, 1; y=0–6 This series of System Test errors may indicate too much light inside the chamber. Make sure the plate carrier door and the front hinged door are properly closed. For models with the dispense module, if the dispense tubes are not connected to the reader, re-install the light shield that shipped with the instrument (or cover the hole with black tape). Restart the reader.
4Fxy	**Fluorescence signal out of range.** x=0, 1; y=0–6 Too low of a reading indicates a light signal problem. Ensure that Gen5 Fluor/Lum wavelengths table matches the actual filter installed in the filter cube.
40xx	**PMT overload well error at <well #xxx>** This error typically means that the fluid in a well has oversaturated the PMT (i.e., the well is too bright). Try lowering the sensitivity value in the read step. **To identify the well:** Wells are counted starting at A1, moving left-to-right, row-by-row. The row and column of the well can be extracted from the well number code by applying the following formula (example uses 8 x 12 geometry, 96-well plate): 1. Convert the ASCII hex string to a decimal equivalent. Ex: “057” indicates 57 hex, yielding a well code of 87 decimal. 2. Row = (well code) / (columns in plate), rounded up to a whole number. Ex: 87/12 = 7.25, indicating row 8 (or H). 3. Column = (well code) - ((row-1) * (columns in plate)). Ex: 87 - ((8 - 1) * 12) = column 3. NOTE: If this code is returned during an area scan, it indicates the scan point corresponding to the row/column equivalent in the currently defined scan map, NOT the actual well where the error occurred.

<table>
<tr><th>Code</th><th>Description and Possible Remedy</th></tr>
<tr><td>4Exy</td><td>
Detector saturated (too much light). Relative Fluorescing Units (RFU) reached (99999).

X = Fluorescence channel

Fluorescence/Luminescence Channel Codes

<table>
<tr><td>Mono optics reference channel</td><td>0</td></tr>
<tr><td>Mono optics PMT channel</td><td>1</td></tr>
<tr><td>Top filter optics reference channel</td><td>2</td></tr>
<tr><td>Top filter optics, top PMT channel</td><td>3</td></tr>
<tr><td>Top filter optics, side PMT channel</td><td>4</td></tr>
<tr><td>Bottom filter optics reference channel</td><td>5</td></tr>
<tr><td>Bottom filter optics PMT channel</td><td>6</td></tr>
</table>

Y = PMT Test Type Code

<table>
<tr><td>Connection Test</td><td>0</td><td>PMT not connected</td></tr>
<tr><td>High Voltage Test</td><td>1</td><td>Failure during test at higher voltage</td></tr>
<tr><td>Low Voltage Test</td><td>2</td><td>Failure during test at lower voltage</td></tr>
<tr><td>Well Overload Test</td><td>5</td><td>Failure during test at well</td></tr>
<tr><td>Background Overload Test</td><td>8</td><td>Failure during background overload test</td></tr>
</table>

OR Y = filter readset

This error can indicate one of several scenarios. It is possibly due to incorrect chemistry, e.g., the fluorescence standards dispensed to the plate exceed expectations.

For models with the dispense module, the internal chamber may require cleaning (contact BioTek TAC).

If a 4E18 error is detected during monochromator-based fluorescence, the luminescence probe may be picking up stray light. Try installing a plug in the filter cube. Restart the reader.
</td></tr>
<tr><td>2D46</td><td>
Fluorescence wavelength not found in table

This error indicates that the wavelength specified in the procedure is not detected in the instrument’s filter table. In Gen5, verify the Fluorescence filter table has the wavelengths loaded into the reader. Compare the contents of the table with the filters installed in the filter cube (see the Gen5 Help system for more information). Restart the reader.
</td></tr>
</table>

Code	Description and Possible Remedy
50xx 510x	**Axis failed to home** Top filter optics, lower filter/mirror slider 03 Top filter optics, upper filter/mirror slider 04 Bottom filter optics, filter/mirror slider 06 Generally, this error indicates the filter cube is not seated properly in the reader. Remove it, ensure each filter or plug is properly positioned and reinstall it securely. Restart the reader.
540x	**Filter cube failed positional verify** Top filter optics, lower filter/mirror slider 03 Top filter optics, upper filter/mirror slider 04 Bottom filter optics, filter/mirror slider 06 Generally, this error indicates the filter cube is not seated properly in the reader. Remove it, ensure each filter or plug is properly positioned and reinstall it securely. Restart the reader.

Code	Description and Possible Remedy
55xy	**<Motor> not homed successfully** xy=axis Axis Codes Carrier X 00 Carrier Y 01 Top probe Z 02 Top filter optics, lower filter/mirror slider 03 Top filter optics, upper filter/mirror slider 04 Bottom probe Z 05 Bottom filter optics, filter/mirror slider 06 Mono optics, excitation filter sector 07 Mono optics, excitation slit wheel 08 Mono optics, emission filter sector 09 Mono optics, emission slit wheel 0A Mono optics, probe changer 0B Excitation monochromator grating 0C Emission monochromator grating 0D Dispenser syringe 1 0E Dispenser syringe 2 0F This error indicates that an axis failed a previous verify function and now needs to be homed. Check for any obstructions that may prevent the carrier, syringes, or filter cube from moving normally. Restart the reader.
570x	**Area Scan matrix too large for perimeter wells** x=0, 1 For some plate type and read probe combinations, it might not be possible to define the entire area scan matrix offered by Gen5 for some perimeter wells, due to the physical limitations of carrier travel. Redefine the area scan to include a smaller matrix or select wells in a different row or column.

Code	Description and Possible Remedy
5A0x	**Plate could not be moved inside** x=0, 1 Make sure the Plate Type defined in the Gen5 Protocol matches the plate you are using. This error can also occur if the plate type is correct but the lid was left on the plate. If you wish to read the plate with a lid on it, create a new plate type in Gen5 with the correct Plate Height. This error can also indicate that the plate is not seated in the carrier correctly. Remove the plate and replace it securely in the carrier. Verify the tip priming trough has not become dislodged. Remove any plates from the carrier and power cycle the instrument to see if the error is resolved.
5B00	**Required carrier in when expected to be outside** Carrier is inside the chamber when it should be outside. This may occur if the read was aborted and "home all axes" not performed. This error can also occur if the carrier is inside and the newly defined plate height is different from the most recently specified plate height. To resolve the error, eject the carrier prior to running the experiment.
70xx	**Motor synchronization error** If at any time it is discovered that a motor is currently on the wrong microstep boundary for a move of a specified microstep size, a motor synch error is flagged. • Verify there is no binding of axis movement. • Turn the instrument off and on to see if the error is repeatable. • Reload the basecode.

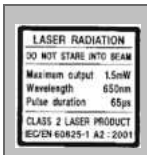
Appendix C

Microplate Barcode Scanner

This appendix contains instructions for installing the optional microplate barcode scanner and specifications for microplate barcode label format and positioning. It also tells you how to set up Gen5 to view and use the scanned barcodes.

Overview

The BioTek external barcode scanner allows you to automate the reading of microplate barcode labels in a robotic system. If a valid barcode is read, the barcode value is automatically passed to Gen5 for storage in the Gen5 experiment.



Warning! Laser Radiation. Do not look directly into the laser beam during operation of the scanner. Serious eye injury may occur if you stare directly into the beam. Please note the warning label on the outside of the scanner's protective cover.

The barcode scanner can be installed on either side of or in front of the reader, depending on which side of the microplate the barcode label is located.

Package Contents

BioTek **PN 1030008** contains:

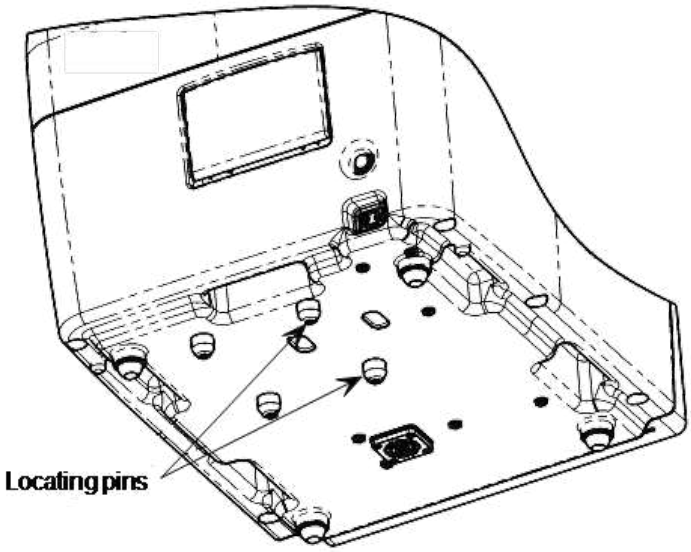
- Barcode scanner assembly: scanner (housed in a protective cover), scanner cable, and mirror attached to a removable mounting bracket
- Scanner alignment bracket

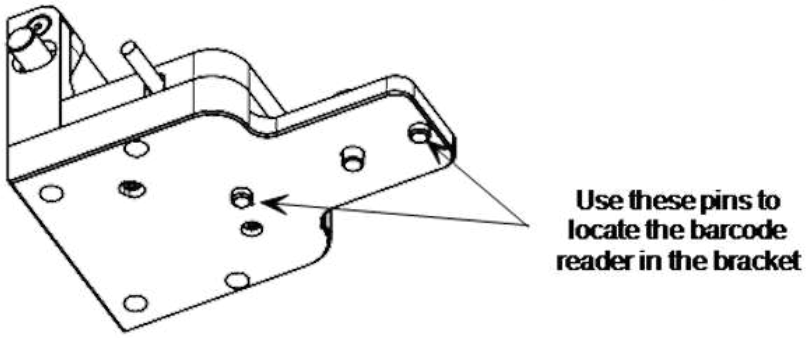
Install the Microplate Barcode Scanner

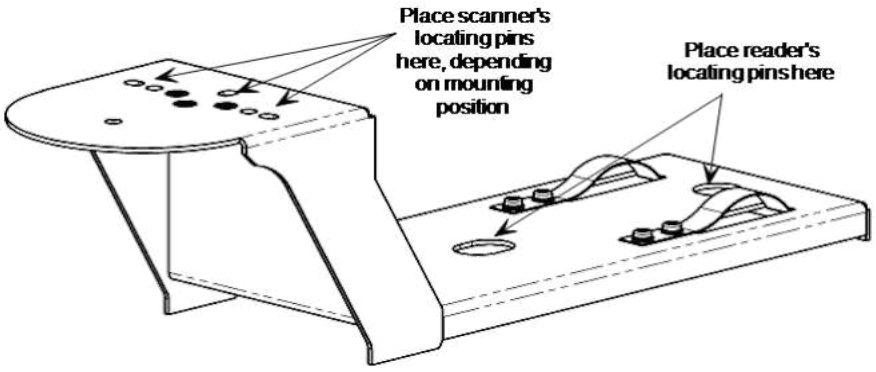
Be very careful not to scratch or damage the scanner's mirror when unpacking or installing the microplate barcode scanner! If you are using a stacker, install the stacker with the interfacing instrument before installing the barcode scanner. **See Chapter 2, Installation**.

The barcode scanner can be placed to the left, the right, or in front of the reader's carrier. Select the mounting position based on the location of the barcode labels on your microplates.

1. Lift the front of the Synergy Neo2, place the mounting bracket under the locating pins, and carefully lower the instrument into the mounting bracket.

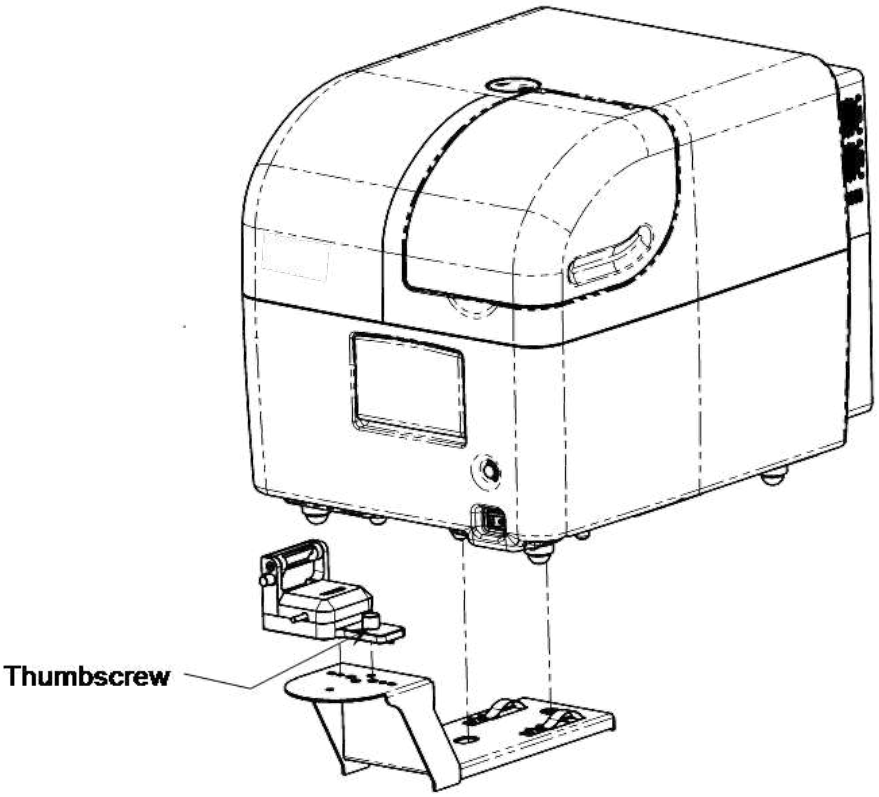




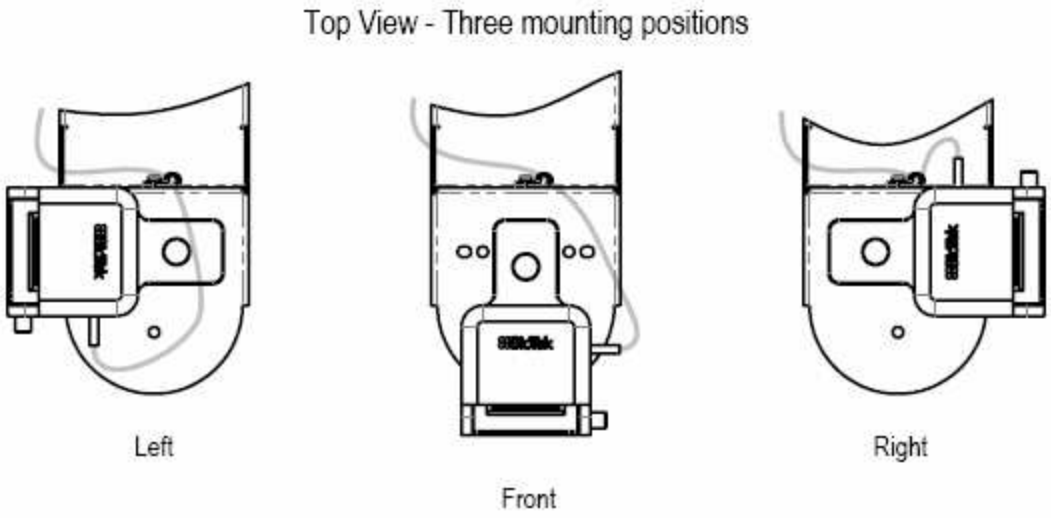


2. Determine the appropriate mounting position for the barcode scanner: left, front, or right.

3. Mount the barcode scanner in the bracket, then tighten the thumbscrew.



4. Plug one end of the communications cable into the barcode reader and connect the other end to the Synergy Neo2.



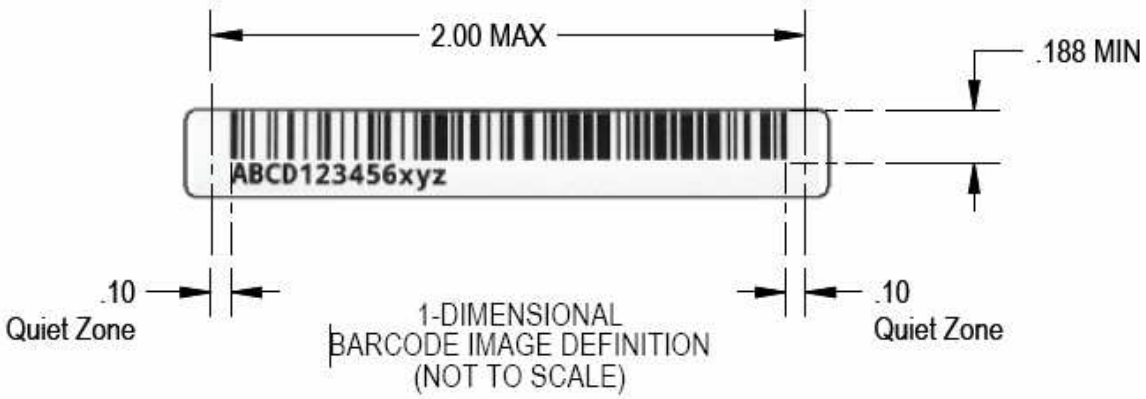
About Barcode Labels

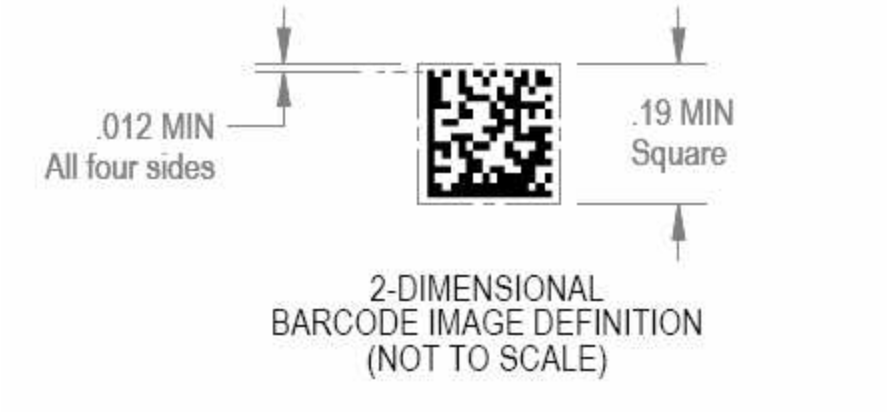
Barcode labels are available for purchase from BioTek, or they may be created using barcode software and label products that meet the following specifications:

- **Label format ("symbology"):** Required formats are:
  - Code 128
  - Code 39
  - Datamatrix
  - PDF 417
  - Codebar
  - UPS
  - QR
  - GS1 Databar
- **Industry Standards:** The labels should be made in accordance with Automation Identification Manufactures (AIM) uniform symbol specification for all codes supported. Label decodability should meet ANSI Specification X2, 182-199 "Bar-Code Print Quality Guideline" for a rating of A/05/880.
- **Label quality:** The labels should be printed on good-quality copier machines or laserjet printers.
- **Label position on plate:** The labels should not extend above or below the edges of the plate, because the plates may stick to one another when they are stacked.

See **Placing a Barcode Label** on page 170 for more information about where to place the label on the microplate.

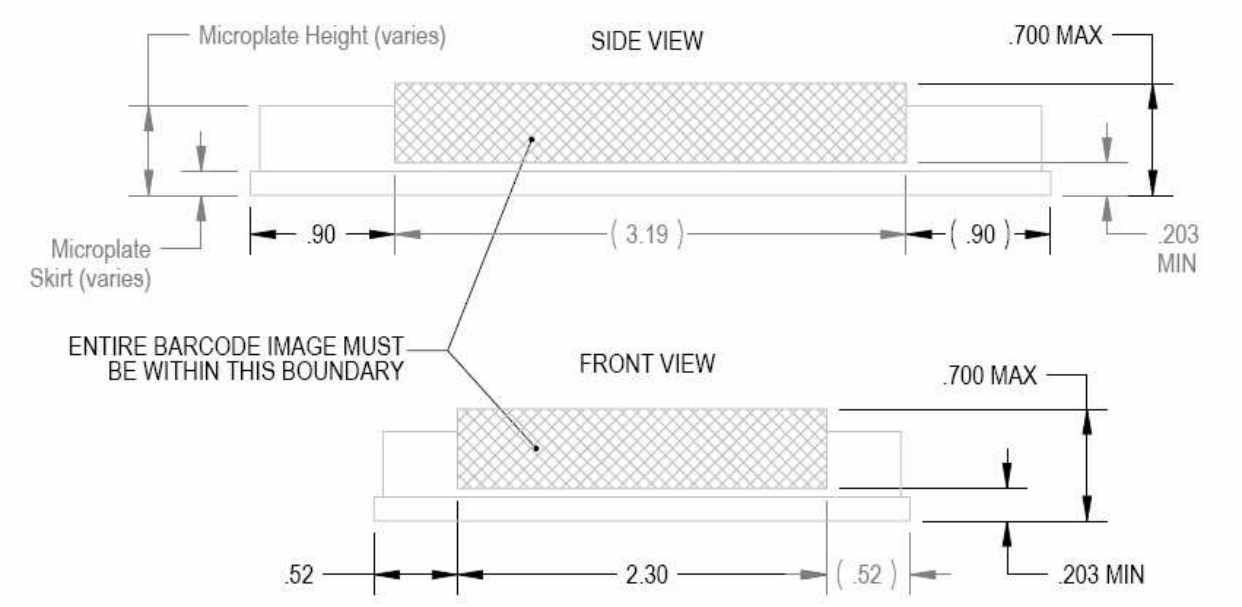
The following figures contain barcode label artwork that may be submitted to a print vendor for creating labels. The areas marked as quiet zones are critical for the reader to locate the barcode image.





Placing a Barcode Label

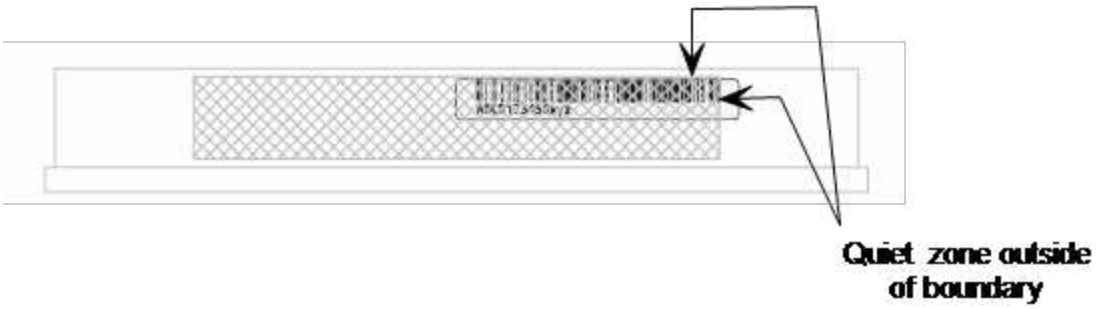
Barcode labels can be located on either the long sides of a microplate or the short side opposite the well A1 location. The next figure shows the location on the microplate where the label must be placed in order to be read properly.



Label Placement Examples

Review the following examples of good label placement:

These examples demonstrate poor placement of the barcode labels:

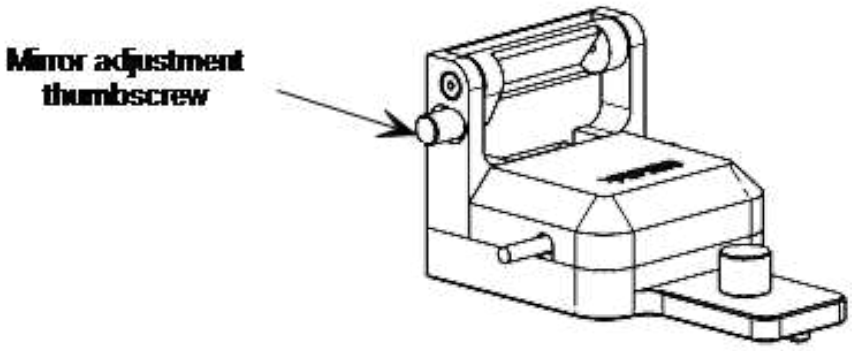


Microplate Scanner Diagnostic Utility

To ensure that the microplate barcode scanner mirror is correctly positioned to scan the barcodes, use the Barcode Scanner Diagnostic utility in Gen5.

1. Place a microplate with a barcode label on the Synergy Neo2 carrier.
2. From the main Gen5 screen, click **System > Instrument Configuration**, select the **Synergy Neo2**, and click **View/Modify > Setup**.

3. On the Barcode Scanner Diagnostic tab, click **Start Diagnostic**. You can see the reflected red light from the laser shining toward the microplate carrier.
4. If necessary, click **Jog Carrier In** to gradually move the carrier so that the barcode label is within the range of the laser light. When the barcode scanner can read the label, the results are displayed in the Reader Response area.
5. If the scanner is unable to read the label, adjust the scanner's mirror position using the mirror adjustment thumbscrew and run the utility again.



6. When the mirror is set correctly, click **Stop Diagnostic**. The barcode scanner is now ready to use.

Appendix D

Sample Reports

This appendix contains sample System Test and Absorbance Plate Test reports for the Synergy Neo2.

Absorbance Test Plate Results

Reader:	Synergy Neo2 (Serial Number: 16060915)
Basecode:	P/N 1350200 (v1.14)
Date and Time:	6/8/2016 2:51:17 PM
Absorbance Plate:	7 Filter Test Plate (P/N 7260522) - S/N 210508
Last Plate Certification:	September 2015
Next Plate Certification Due:	September 2016
User:	Administrator
Comments:	

Peak Absorbance Results

Well	C6	C6	C6	C6
Reference	279	361	418	643
Tolerance	3	3	3	3
Read	280	362	419	643
Result	PASS	PASS	PASS	PASS

Alignment Results

Wells	A1	A12	H1	H12
Read	0.000	0.000	0.000	0.000
Tolerance	0.015	0.015	0.015	0.015
Result	PASS	PASS	PASS	PASS

Wavelength = 450 nm

Accuracy Results

Wells	C1	E2	G3	H6	F5	D4
Reference	0.143	0.580	1.082	1.645	1.919	2.496
Min Limit	0.120	0.548	1.040	1.592	1.861	2.376
Max Limit	0.166	0.612	1.124	1.698	1.977	2.616
Read 1	0.140	0.577	1.083	1.649	1.919	2.500
Result	PASS	PASS	PASS	PASS	PASS	PASS

Repeatability Results

Wells	C1	E2	G3	H6	F5	D4
Read 1	0.140	0.577	1.083	1.649	1.919	2.500
Min Limit	0.133	0.566	1.067	1.628	1.895	2.420
Max Limit	0.146	0.588	1.099	1.670	1.943	2.580
Read 2	0.140	0.578	1.084	1.650	1.919	2.499
Result	PASS	PASS	PASS	PASS	PASS	PASS

Wavelength = 630 nm

Accuracy Results

Wells	C1	E2	G3	H6	F5	D4
Reference	0.169	0.589	1.099	1.673	1.795	2.337
Min Limit	0.146	0.557	1.057	1.620	1.739	2.224
Max Limit	0.192	0.621	1.141	1.726	1.851	2.450
Read 1	0.166	0.584	1.094	1.667	1.793	2.335
Result	PASS	PASS	PASS	PASS	PASS	PASS

Repeatability Results

Wells	C1	E2	G3	H6	F5	D4
Read 1	0.166	0.584	1.094	1.667	1.793	2.335
Min Limit	0.159	0.573	1.078	1.645	1.770	2.260
Max Limit	0.172	0.595	1.110	1.688	1.815	2.410

Read 2	0.166	0.584	1.094	1.667	1.793	2.335
Result	PASS	PASS	PASS	PASS	PASS	PASS

Wavelength = 750 nm

Accuracy Results

Wells	C1	E2	G3	H6	F5	D4
Reference	0.148	0.474	0.881	1.339	1.273	1.657
Min Limit	0.125	0.445	0.843	1.292	1.228	1.604
Max Limit	0.171	0.503	0.919	1.386	1.318	1.710
Read 1	0.147	0.472	0.878	1.335	1.270	1.651
Result	PASS	PASS	PASS	PASS	PASS	PASS

Repeatability Results

Wells	C1	E2	G3	H6	F5	D4
Read 1	0.147	0.472	0.878	1.335	1.270	1.651
Min Limit	0.141	0.462	0.865	1.316	1.252	1.630
Max Limit	0.154	0.481	0.892	1.353	1.288	1.673
Read 2	0.147	0.472	0.878	1.335	1.270	1.651
Result	PASS	PASS	PASS	PASS	PASS	PASS

Reviewed/Approved By: _____ Date: _____

For Technical Support

In the U.S.:
BioTek Instruments, Inc.
Tel: 800 242 4685
Fax: 802 654 0638

In Europe:
BioTek Instruments GmbH
Tel: 49(0)7136-9680
Fax: 49(0)7136-968-111

All Others:
Tel: 802 655 4040
Fax: 802 654 0638

email: TAC@biotek.com
Product support center: http://www.biotek.com/service

Absorbance Test Plate Results

Reader:	Synergy Neo2 (Serial Number: 16060915)
Basecode:	P/N 1350200 (v1.14)
Date and Time:	6/8/2016 2:57:23 PM
Absorbance Plate:	6 Filter Test Plate (P/N 7260551) - S/N 283921
Last Plate Certification:	October 2015
Next Plate Certification Due:	October 2016
User:	Administrator
Comments:	

Alignment Results

Wells	A1	A12	H1	H12
Read	0.000	0.000	0.000	0.000
Tolerance	0.015	0.015	0.015	0.015
Result	PASS	PASS	PASS	PASS

Wavelength = 340 nm

Accuracy Results

Wells	C1	E2	G3	H6	F5	D4
Reference	0.651	0.642	0.925	0.927	1.263	1.273
Min Limit	0.618	0.609	0.887	0.888	1.218	1.228
Max Limit	0.684	0.675	0.964	0.966	1.308	1.318
Read 1	0.658	0.646	0.931	0.934	1.272	1.281
Result	PASS	PASS	PASS	PASS	PASS	PASS

Repeatability Results

Wells	C1	E2	G3	H6	F5	D4
Read 1	0.658	0.646	0.931	0.934	1.272	1.281
Min Limit	0.646	0.634	0.917	0.919	1.254	1.263
Max Limit	0.669	0.657	0.946	0.948	1.290	1.299
Read 2	0.658	0.646	0.931	0.934	1.273	1.281
Result	PASS	PASS	PASS	PASS	PASS	PASS

Reviewed/Approved By: _____ Date: _____

For Technical Support

In the U.S.:
BioTek Instruments, Inc.
Tel: 800 242 4685
Fax: 802 654 0638

In Europe:
BioTek Instruments GmbH
Tel: 49(0)7136-9680
Fax: 49(0)7136-968-111

All Others:
Tel: 802 655 4040
Fax: 802 654 0638

email: TAC@biotek.com
Product support center: http://www.biotek.com/service

Gen5 System Test Report

Reader:	Synergy Neo2 (Serial Number: 16060915)
Basecode:	P/N 1350200 (v1.14)
Gen5 Version:	2.09.1
Date and Time:	6/8/2016 2:45:26 PM
User:	Administrator
Company:	
Comments:	

Test Results
SYSTEM TEST PASS

Operator ID: _____

Notes: _____

_____

SYSTEM SELF TEST

1350200 Version 1.14 16060915 S1 S2 S3
1011 1000 0000
MBA

Voltage Reference Test	Min	Low	High	Max
Mono System Flash	1416	1751	2103	3089
Top Filter Flash	2332	2915	3498	
Bottom Filter Flash	2346	2930	3514	
Switched 24V Power	1875			

ABSORBANCE

Optics Test	Ref	Meas	Gain	Resets	R/G
#1:230			1.67	1	0.597
Tested			2.02	1	0.496
Light	13537	39855			
Dark	10609	10632			
Delta	2928	29223			
#2:285			2.70	4	1.483
Tested			2.94	4	1.358
Light	13140	39784			
Dark	10616	10659			
Delta	2524	29125			
#3:405			2.03	8	3.936
Tested			2.21	8	3.623
Light	12998	39974			
Dark	10593	10649			
Delta	2405	29325			
#4:630			2.07	8	3.873
Tested			2.35	8	3.404
Light	12752	39909			
Dark	10596	10651			
Delta	2156	29258			
#5:800			2.49	4	1.608
Tested			1.62	2	1.234
Light	12651	39981			
Dark	10584	10632			
Delta	2067	29349			

#6:999			2.49	2	0.804
Tested			2.03	1	0.492
Light	12683	39712			
Dark	10608	10631			
Delta	2075	29081			

Noise Test	Ref	Meas
Gain 1.00		
Max	10571	10619
Min	10571	10618
Delta	0	1
Gain 2.03		
Max	10610	10631
Min	10609	10630
Delta	1	1

FLUORESCENCE/LUMINESCENCE

Monochromator System			
Bias current offset	0.0	counts	PASS
Offset voltage	1664	counts	PASS
750V measurement	159.2	counts	PASS
750V noise	38	counts	
750V offset	1678	counts	
Reset offset	1717	counts	
Reference bias	0.0	counts	PASS
Reference offset	10543	counts	PASS
Reference noise	0.2	counts	PASS

Top Filter System - Top			
Bias current offset	-1.1	counts	PASS
Offset voltage	1665	counts	PASS
750V measurement	543.5	counts	PASS
750V noise	221	counts	
750V offset	1693	counts	
Reset offset	1729	counts	
Reference bias	-2.6	counts	PASS
Reference offset	10562	counts	PASS
Reference noise	0.2	counts	PASS

Top Filter System - Side			
Bias current offset	-2.0	counts	PASS
Offset voltage	1653	counts	PASS
750V measurement	98.5	counts	PASS
750V noise	25	counts	
750V offset	1660	counts	
Reset offset	1716	counts	

Bottom Filter System			
Bias current offset	-0.6	counts	PASS
Offset voltage	1669	counts	PASS
750V measurement	158.8	counts	PASS
750V noise	38	counts	
750V offset	1680	counts	
Reset offset	1723	counts	
Reference bias	0.9	counts	PASS
Reference offset	10564	counts	PASS
Reference noise	0.2	counts	PASS

Filter Fluorescence

Top Probe			
Reference	400V	500V	600V
Gain	1.00	1.00	1.00

Light	12699	14206	16003
Dark	10562	10562	10562
Delta	2137	3644	5441

Bottom Probe

Reference	400V	500V	600V
Gain	1.00	1.00	1.00
Light	12268	13488	14958
Dark	10565	10565	10565
Delta	1703	2923	4393

Mono Fluorescence - Optics Test - 500V

Top Probe

Bandpass	17nm		40nm	
Sensitivity:42	Ref	Meas	Ref	Meas
#1:300				
Light	14936	4270	15754	8023
Dark	10631	1663	10587	1663
Delta	4305	2607	5167	6360
Max	4396	2645	5271	6458
Min	4195	2544	5063	6259
StdDev	56	36	56	54
#2:485				
Light	27204	11894	28436	21522
Dark	10631	1663	10588	1663
Delta	16573	10231	17848	19859
Max	16862	10361	18068	20048
Min	16174	10061	17778	19746
StdDev	231	105	90	93

Bottom Probe

Bandpass	17nm		40nm	
Sensitivity:38	Ref	Meas	Ref	Meas
#1:300				
Light	14919	2611	15729	4020
Dark	10631	1663	10588	1662
Delta	4288	948	5141	2358
Max	4350	965	5249	2385
Min	4191	916	5013	2302
StdDev	48	14	67	24
#2:485				
Light	27283	14459	28480	25465
Dark	10631	1663	10588	1661
Delta	16652	12796	17892	23804
Max	16816	12892	18080	23970
Min	16481	12707	17731	23583
StdDev	117	67	105	109

CALIBRATION

Carrier - Top Mono Fluorescence

Upper Left	x= -98	y= 8597
Lower Left	x= -100	y= 2381
Lower Right	x= 9663	y= 2379
Upper Right	x= 9664	y= 8598
Delta 1	-98 - -100=	+2
Delta 2	9664 - 9663=	+1
Delta 3	8598 - 8597=	+1
Delta 4	2379 - 2381=	-2

Carrier - Bottom Mono Fluorescence

Upper Left	x= 1933	y=10487
Lower Left	x= 1931	y= 4277
Lower Right	x=11697	y= 4275

```
    Upper Right       x=11700   y=10489
    Delta 1            1933 -  1931=   +2
    Delta 2           11700 - 11697=   +3
    Delta 3           10489 - 10487=   +2
    Delta 4            4275 -  4277=   -2

Carrier - Absorbance
    Upper Left        x= 1899   y= 8588
    Lower Left        x= 1897   y= 2375
    Lower Right       x=11662   y= 2373
    Upper Right       x=11666   y= 8588
    Delta 1            1899 -  1897=   +2
    Delta 2           11666 - 11662=   +4
    Delta 3            8588 -  8588=   +0
    Delta 4            2373 -  2375=   -2

Carrier - Top Filter Fluorescence
    Upper Left        x=-3659   y= 6621
    Lower Left        x=-3667   y=  414
    Lower Right       x= 6105   y=  405
    Upper Right       x= 6104   y= 6622
    Delta 1           -3659 - -3667=   +8
    Delta 2            6104 -  6105=   -1
    Delta 3            6622 -  6621=   +1
    Delta 4             405 -   414=   -9

Carrier - Bottom Filter Fluorescence
    Upper Left        x=-3161   y=10042
    Lower Left        x=-3158   y= 3835
    Lower Right       x= 6604   y= 3830
    Upper Right       x= 6610   y=10048
    Delta 1           -3161 - -3158=   -3
    Delta 2            6610 -  6604=   +6
    Delta 3           10048 - 10042=   +6
    Delta 4            3830 -  3835=   -5

Carrier - Injectors
    Upper Left        x= 2197   y= 6621
    Lower Left        x= 2189   y=  414
    Lower Right       x=11961   y=  405
    Upper Right       x=11960   y= 6622
    Delta 1            2197 -  2189=   +8
    Delta 2           11960 - 11961=   -1
    Delta 3            6622 -  6621=   +1
    Delta 4             405 -   414=   -9

Carrier - Test Sensors
    Middle Sensor      23151
    Tested             23151
    Delta                 +0

Top Probe Height       26.60 mm

Bottom Probe Height    14.09 mm

Cube Sliders
    Top Lower           6440
    Top Upper           7184
    Bottom              6372

Excitation Monochromator
    Slit Wheel          8568
    Probe Changer       4172
```

```
    Backlash              36
  305LP Edge           -942.15
  Tested               -943.54
  Top Fluorescence     B=+0.00021273 C=+0.06942856
  Bottom Fluorescence  B=+0.00045812 C=+0.30668175
  Absorbance           B=+0.00069251 C=-0.21832025

Emission Monochromator
  Slit Wheel           8656
  Probe Changer        4128
    Backlash             28
  629nm Peak            +27.00
  Tested                +26.61
  Top Fluorescence     B=+0.00037734 C=-1.17780030
  Bottom Fluorescence  B=-0.00088149 C=-0.58775091

Cube Scanners           Min       Max
  Top Lower              32        32
  Top Upper              27        27
  Bottom                 26        26

Alpha Laser Module     1030201  Version 2.05.0
  Current DAC          114
  PD Threshold         0000,0118
  Status               0005                          *TEC DISABLED*


INCUBATION

Temperature Setpoint: 37.0  Current Average: 37.0   A/D Test: PASS

Zone 1: 37.0  Min: 36.9  Max: 37.1   Range: PASS  Thermistor: PASS
Zone 2: 36.9  Min: 36.9  Max: 37.1   Range: PASS  Thermistor: PASS
Zone 3: 36.9  Min: 36.9  Max: 37.1   Range: PASS  Thermistor: PASS
Zone 4: 37.0  Min: 36.9  Max: 37.0   Range: PASS  Thermistor: PASS

0000

Dispenser 1: 005.3,010.4,020.4,040.1,080.0,199.7
Dispenser 2: 005.3,010.3,020.5,040.2,080.1,200.2

Excitation monochromator serial number: 228816
Emission monochromator serial number:   228817


Reviewed/Approved By: _____   Date: _____

For Technical Support

In the U.S.:
BioTek Instruments, Inc.
     Tel: 800 242 4685
     Fax: 802 654 0638
In Europe:
     BioTek Instruments GmbH
     Tel: 49(0)7136-9680
     Fax: 49(0)7136-968-111

All Others:
     Tel: 802 655 4040
     Fax: 802 654 0638

email: TAC@biotek.com
Product support center: http://www.biotek.com/service
```